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## A FLUID MEDIUM FOR THE ENCYSTATION OF *ENDAMOEBA HISTOLYTICA* UNDER REDUCED ATMOSPHERIC PRESSURE*

LUCILE K. ZUCKERMAN AND HENRY E. MELENEY

Department of Preventive Medicine, College of Medicine, New York University

In cultivating *Endamoeba histolytica* in Erlenmeyer flasks for the purpose of obtaining large crops of cysts for experimental purposes we found, over a period of years, that there were periods of weeks or months when very poor crops of cysts were obtained even though good multiplication of the amoebae occurred. The media employed for encystation was basically the same as that described by Frye and Meleney (1935) for the cultivation of *E. histolytica* in Erlenmeyer flasks, although modifications were employed from time to time. A solid egg-Ringer base was overlayed with horse serum-Ringer or a solution of liver extract or Ringer's solution or normal saline, and an appropriate amount of rice flour was added. The inoculum was always the sediment from several tubes of cultures of *E. histolytica* without rice.

In addition to the irregularity of cyst production in such flasks, particles of coagulated egg, which were carried over in the washing procedure when the cysts were removed from the flasks, interfered with the separation of the cysts from the accompanying bacteria and rice. Various attempts were made to develop a completely fluid medium which would produce good crops of cysts regularly in flasks. The present paper describes a medium which has proved satisfactory.

### PRE-ENCYSTATION MEDIUM

The strains of *E. histolytica* from which cysts are to be obtained are kept in tube cultures containing slants of Cleveland and Collier's Difco *Endamoeba* medium stiffened by raising the agar content to 2% as recommended by Kessel et al (1944). The slants are overlayed with inactivated horse serum-Ringer 1–10. This medium is better than egg-Ringer slants because no particles of the solid slant break off and are carried over into the encystation medium.

### ENCYSTATION MEDIUM

The stock medium is composed of Cerophyl[1] extracted in our CPR medium. The CPR medium consists of cholesterol, one part per million, and Difco proteose peptone 0.5% in Ringer's solution. Cerophyl is suspended in this medium in a

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[1] Manufactured by Cerophyl Laboratories, Inc., Kansas City, Missouri. Cerophyl is a powder containing the dry leaves of young cereal plants. It is said to be rich in carotene, ascorbic acid, riboflavin, Vitamin K, grass juice factor and chlorophyl.

concentration of 2% and the mixture is autoclaved at 15 pounds pressure for 20 minutes. After cooling it is filtered through filter paper. The filtrate is a slightly cloudy greenish-blue liquid which cannot be clarified by further passing through filter paper. Seitz filtration is not employed because it was found to decrease the nutritive quality of the medium. The medium is reautoclaved and stored. A few hours before use it is dispensed in 50-ml amounts in 250-ml Erlenmeyer flasks, and is again autoclaved. Adjustment of the hydrogen-ion concentration is not necessary. Just before inoculation 5 ml of inactivated horse serum and 0.02 gram of especially prepared rice starch are added. It has been found advantageous to use pure rice starch in the form of separate, minute granules so that as little residue as possible will remain after maximum encystation is produced. The rice starch is prepared by digesting 100 grams of Difco rice powder with 2 grams of Armour's trypsin powder in 1000 ml of distilled water; 10 ml of toluene is added and the pH is adjusted to 7.0–7.2. The mixture is incubated at 37° C for 2–3 days, the pH being adjusted again if necessary after the first 24 hours. When microscopic examination shows that the rice starch is apparently free of protein material, the mixture is filtered through six double layers of cheese cloth. The filtrate, consisting of a practically pure suspension of individual starch granules, is washed three times in distilled water and dried with acetone. One gram of this rich starch is sterilized in a 250-ml Erlenmeyer flask in a hot air oven at 150° C for 90 minutes, after which 50 ml of the sterile Cerophyl extract is added to it. After thorough shaking, 1 ml of this suspension, which contains 0.02 gram of rice starch, is added to each encystation flask. The addition to the minimum amount of rich starch required to produce maximum encystation was suggested by Kessel et al (1944) for tube cultures, and the above amount was determined by us for flasks. This was done in order to minimize the amount of rice which would have to be separated from the cysts by flotation in zinc sulphate in our attempts to produce bacteria-free cysts (Snyder and Meleney, 1941).

### INOCULUM

The sediment of three tubes of pre-encystation cultures of *E. histolytica* without rice, showing moderate growth after 48 to 72 hours is pooled and seeded into each encystation flask, and the flask gently rotated to disperse the amoebae and rice starch.

### EVACUATION OF AIR AND INCUBATION

After inoculation the cotton plug of the flask is replaced by a sterile one-hole rubber stopper fitted with a glass tube and rubber tube with a hose cock, and sealed with a small amount of sterile glycerine to keep the flask airtight. The air in the flask is then evacuated by an oil pump to a pressure of 40 mm of mercury, the flask being gently rotated during this process. It is then incubated at 37° C for about 70 hours when it is opened and examined for maturity of the cysts. This is usually the time of maximum production of cysts. If many of the cysts are immature at this time the flask is incubated aerobically for 3–4 hours and reexamined.

### RESULTS

By this method of cultivation we have obtained excellent crops of cysts regularly for over a year. In over 100 flasks the average number of cysts from each flask

has been close to 4 million. The encysted cultures can be stored in the flasks at refrigerator temperature under either normal or reduced atmospheric pressure for at least two weeks without apparent injury to the cysts. After the cysts are washed, however, we have found it necessary to complete our manipulations and transfer into new media on the same day in order to obtain excystation.

This encystation medium will not support the multiplication of *E. histolytica* in flasks under aerobic conditions, although under the reduced atmospheric pressure a very abundant multiplication is obtained. We have not arrived at a satisfactory explanation of this difference. One might expect that more profuse bacterial multiplication under aerobic incubation would provide the necessary anerobiosis for the amoebae. It is possible, however, that the medium does not contain sufficient nutriment to produce adequate multiplication of bacteria, and that the medium absorbs enough oxygen from its broad surface in the flask to prevent the development of satisfactory anaerobic conditions for the amoebae. These observations emphasize further the anaerobic character of *E. histolytica.*

SUMMARY

A fluid medium is described for the production of consistently large crops of cysts of *E. histolytica* in Erlenmeyer flasks. The medium consists of Cerophyl extracted in a solution of peptone and cholesterol in Ringer's solution with the addition of horse serum and rice starch. The cultures must be incubated at reduced atmospheric pressure in order to produce multiplication of the amoebae preliminary to cyst formation.

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# THE ISOLATION OF MICROFILARIAE FROM BLOOD FOR USE AS ANTIGEN*

MYRON B. FRANKS[1] AND NORMAN R. STOLL[2]

United States Naval Medical Research Unit Number Two and The Rockefeller Institute for Medical Research, Princeton, New Jersey

While it has been known for some time that extracts of adult filarids are capable of eliciting intradermal reactions and can also be used in serological tests (Taliaferro and Hoffman, 1930; Fairley, 1931), less attention has been devoted to the use of filarid embryos as test antigen.[3]

In the development of a technique of isolating microfilariae from the blood of dogs infected with *D. immitis,* we had two objectives: that the organisms be freed in a viable condition from the circulating blood elements and from the soluble blood substances, and that the yield of microorganisms be sufficiently high to make the process applicable to studies on human filariasis. Both of these objectives were achieved.

Our best results were obtained as follows: Sterile technic was used throughout. Blood was drawn into a flask containing 5% sodium citrate in saline until the citrate solution and the blood were in the ratio of 1 : 4. The citrated blood was centrifuged for 30 minutes at 2500 RPM, after which the plasma supernate was removed for separate processing. The WBC layer was drawn off and discarded. The packed red cell and microfilarial mass were brought to original blood volume with a 4 : 1 saline-citrate solution (normal physiological saline 4 parts and 3.8% sodium citrate one part), and the red cells were then hemolyzed with saponin. Approximately 1.0 ml of a 15% solution of saponin in physiological saline was required for each 15 ml of original blood volume. Care was used not to add saponin beyond the amount required for complete hemolysis as an excess caused a high mortality among the microfilariae. The preparation, under the microscope, at this stage showed the actively motile microfilariae and red blood cells shadows in the hemoglobin-tinged diluent.

The hemolyzed blood was centrifuged for 30 minutes at 2500 RPM after which the supernate, now practically free of larvae, was discarded. The stroma-microfilaria sediment was washed two or three times with saline-citrate solution to remove as much of the saponin as possible. The supernates were discarded. Most of the microfilariae were caught in the stroma sediment and their release required considerable effort and time. It was best accomplished by adding saline-citrate solution to the packed mixture of stroma and microfilariae, shaking vigorously to loosen the microfilariae, centrifuging at high speed for 30–60 seconds to bring down the stroma, recentrifuging the stroma-free supernate to recover its microfilarial con-

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* The opinions and assertions contained herein are the private ones of the writers, and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

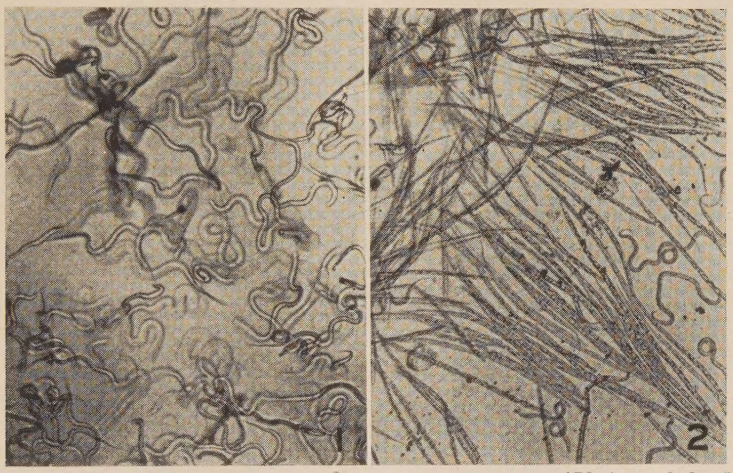
[1] Lieut. MC(S) USNR.

[2] Lieut. H(S) USNR.

[3] The recent report of Oliver-Gonzáles and Bercovitz (1944) came to our attention after our work was terminated and this report prepared.

tent, and resuspending the stroma with more saline-citrate solution, shaking and repeating the process. A dozen washings of the stroma were sometimes needed to secure a good yield. The stage of the process requiring the shaking and the short period of centrifugation at high speed was best carried out in rubber-capped 250-ml pyrex centrifuge bottles.

The microfilariae thus isolated were in a concentration 200 or more times that of the original blood. They were then washed with physiological saline (if they were to be stored in plasma) or Tyrode solution and stored in diluted plasma, serum or Tyrode solution. In storing microfilariae so as to retain their viability, they were best kept dispersed in a shallow layer in an Erlenmeyer flask and refrigerated. Under these conditions they have routinely remained viable for two weeks or more. Fig. 1 shows a drop of living microfilariae in saline photographed with 1/200 second exposure by flash bulb.



*(Photograph by J. A. Carlile)*

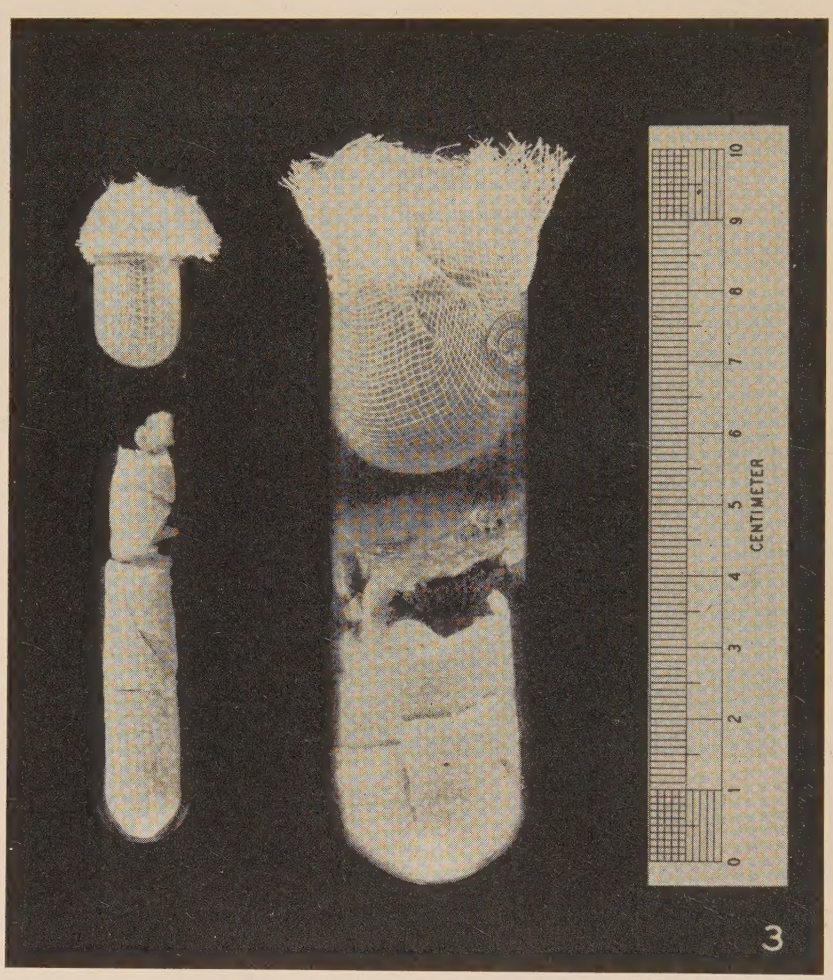
FIG. 1. Living microfilariae in saline, after isolation from blood, photographed with 1/200-second exposure by flash bulb. (100×.)

FIG. 2. The "lining up" of living microfilariae in a thin film preparation in saline photographed with 1/200 exposure by flash bulb. (100×.)

As noted above the whole process was carried out under sterile conditions and as completely as possible in the cold. This included not only refrigeration of the various diluting solutions and sublots of blood, but immersion of flasks, tubes, centrifuge cups, etc., in iced water. To secure a yield of 80 or more per cent of the microfilariae from a liter of blood required 48–72 man hours of continuous processing. When work has been begun with the saponized hemolysate, there does not appear to be any stage at which the process may be stopped and the larvae safely stored.

To prepare the microfilariae in dry form they were further concentrated by centrifugation and washed several times with saline. As much as possible of the last saline wash was drawn off and the larvae resuspended in a small amount of distilled water, transferred to a tube of known weight and then lyophilized. Additional distilled water washes were not used in order to avoid the death of microfilariae and the leaching out of antigenic materials under hypotonic conditions. The microfilariae dried as a white, fluffy, talc-like substance (Fig. 3). Calculation from

several specimens indicated that there were 400–500 million in a gram, dry weight. They were stored at room temperature in a desiccator. Upon rewetting, the dry twisted sticks of larvae straightened and filled out to assume an almost life-like appearance (Figs. 4–5).

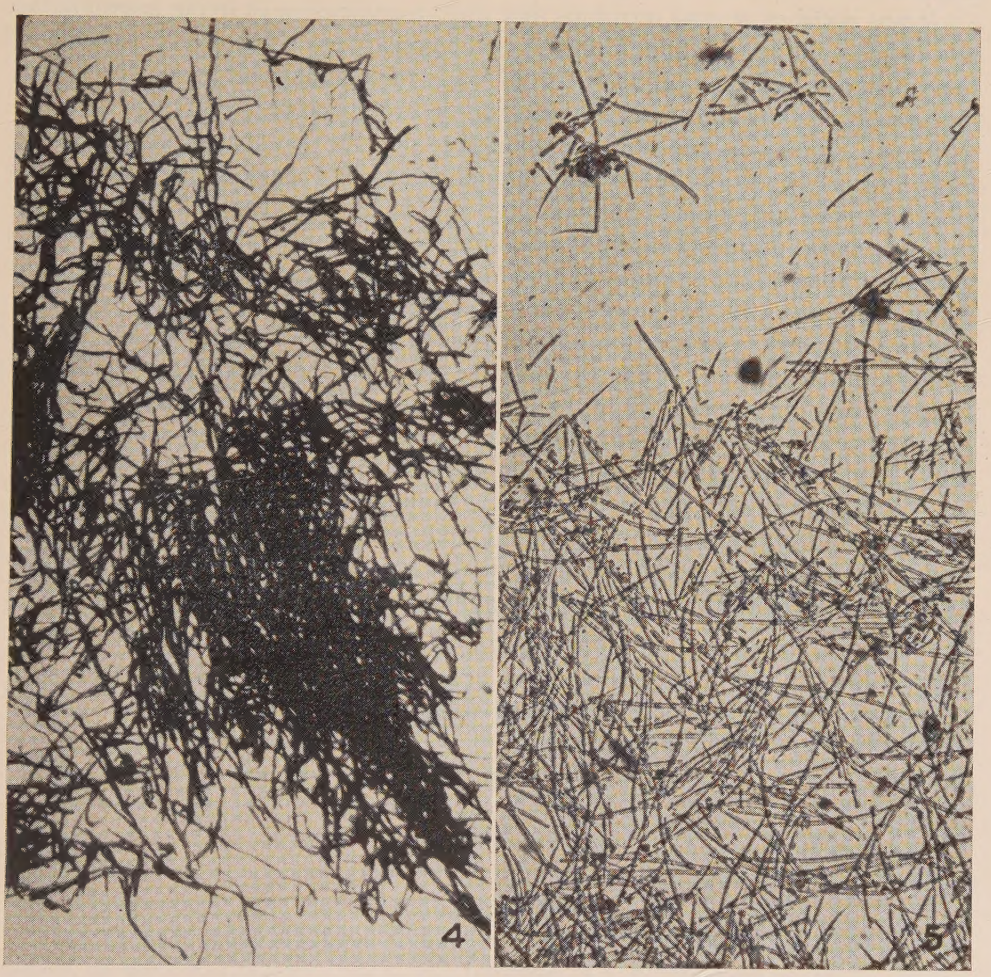



*(Photograph by J. A. Carlile)*

FIG. 3. Tubes of lyophilized microfilariae. Each tube shows approximately 50 million, the apparent difference in mass being due to the volume of water present when frozen.

Conditions beyond control prevented adequate serological testing of the microfilariae as a reagent but limited observations suggest their antigenic activity. The antigen was prepared on a dry weight basis with an initial dilution of 1/100 in physiological saline. The dried microfilariae were ground under sterile conditions, physiological saline was added, and the suspension kept at room temperature (26° C.) for several hours with frequent shaking, left overnight in the refrigerator and then centrifuged for 15 minutes at 3000 RPM. The supernate was used as the test antigen.

Precipitin tests were made with the sera of 20 dogs with varying degrees of microfilaremia and of 8 men known to have filariasis. All necessary control tests, negative serum, etc., were performed. The precipitin tests on the dog sera were positive at dry weight dilutions of antigen ranging from 1/1600 to 1/25,000. In many instances animals with large numbers of circulating microfilariae demonstrated low titres or gave negative reactions. The human sera reacted within the same range as the dog serum. Parallel tests set up with extracts made from dried adult




*(Photograph by J. A. Carlile)*

FIG. 4. Photomicrograph of wisps of dry microfilariae, photographed dry by transmitted light. (100×.)

FIG. 5. Portion of preparation photographed in Fig. 4 upon rewetting with water.

*D. immitis* gave results similar to those with the microfilarial antigen but at a slightly lower titre. Controls were uniformly negative. Tests made with stroma contaminated antigen gave cross reaction with normal serum to a dilution of 1/1600.

In connection with the use of an extract derived from microfilariae, attention should be drawn to the difficulty of thoroughly extracting them. Besides dry grinding in a mortar, we also tried trituration in the TenBroeck grinder and after liquid air freezing. Microscopical examination of the debris following the use of these

methods showed that the microfilariae are particularly resistant to complete fragmentation. Some remained whole and apparently undamaged, others were in short cylindrical pieces with cuticle intact. Occasionally the latter would show gaping or splintered ends, but with the majority of the cells apparently undamaged. The failure of the methods used to secure the marked disintegration expected of vigorous grinding undoubtedly indicated that the extracts secured were less potent than the microfilariae are capable of furnishing.

It was noted on several occasions that when a suspension of living microfilariae was added to the serum of an infected dog, the preparation rimmed with vaseline to prevent drying and then set aside for an hour, the microfilariae gradually slowed their active wiggling and arranged themselves in orderly rows like so many matches in a box. So long as they were not allowed to dry out they maintained their viability for many hours, stayed in close approximation in a single-layer row and exhibited a gentle undulating movement. (See Fig. 2.) We thought this might be an agglutination phenomenon and checked the observation by hanging drop and thin film preparation on the serum of 26 dogs infected with *D. immitis* and 8 men known to have filariasis. In the hanging drop preparation no "lining up" of the microfilariae was noted in any of the infected serum, control serum, saline or Tyrode solution, except in those instances when the drop was not properly centered and formed a thin film at the edge of the hollow of the slide and the cover slip. As a rule the microfilariae would settle to the lowest part of the drop and maintain their activity for many hours. In the thin film preparations the "lining up" phenomenon was demonstrated not only in the immune canine and human serum but also in normal serum, physiological saline and Tyrode solution. It was, therefore, concluded that the "lining up" of the microfilariae we observed was apparently a surface tension phenomenon rather than an agglutination reaction.

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# THE STRUCTURE OF THE COMMON INTESTINAL TRICHOMONAD OF MAN

HAROLD KIRBY

Department of Zoology, University of California

In this paper an account will be given of the same trichomonad about which I published a note reporting the results of dark-field observation (Kirby, 1943). An abstract of that note by Wenyon (1944) in the Tropical Diseases Bulletin, stated that I considered the flagellate to be a species distinct from others found in the human intestine. On the contrary, I follow Wenrich (1944b) in his opinion that there is one species that is commonly found in the intestine of man; and, in fact, there are grounds for surmising that there is a possibility that all accounts of trichomonads from that situation, except that of *Tritrichomonas fecalis,* have actually dealt with the one species. A definitive description of that species has recently been written by Wenrich (1944a). This article will report certain features of the structure of the flagellate revealed by procedures different from those included in his studies and the many others that have been published.

Wenrich made his studies chiefly on specimens in fecal smears stained with Heidenhain's iron haematoxylin; and on flagellates, grown in cultures, that were dried after exposure to osmic acid fumes and stained by Giemsa's method. The two strains of trichomonads described in this paper were grown in culture in Ringer's solution plus one-eighth horse serum over an egg slant. One strain was derived from a stool specimen from a person in California in May, 1943. The other strain was sent in 1944 from the Army Medical School to the School of Tropical Medicine at the U. S. Naval Hospital, Treasure Island, California. Film preparations from these cultures were fixed in Hollande's cupric picroformal and impregnated by Bodian's protein-silver technique. The procedure was as follows: 1% aqueous protargol with 0.5 g copper wire in 10 cc (the wire coiled at the bottom of the Columbia cover glass staining dish) 12–48 hr at 37° or room temperature; washing; 1% hydroquinone in 5% sodium sulfite, 5–10 min; washing; 1% aqueous gold chloride, 2–4 min; washing; 2% aqueous oxalic acid, 2–5 min; washing; 5% aqueous sodium thiosulfate, 5–10 min; thorough washing, dehydration, and clearing. Observations have also been made by dark-field illumination.

The value in protozoological technique of silver impregnation by activated protargol, as used by Bodian (1937), was reported by Cole and Day (1940). Before their statement was published, the striking result obtainable in certain flagellates by the method was called to my attention by Dr. L. R. Cleveland.

Credit is due to Mrs. Marietta Voge for assistance in the study and for making the drawings; to Mr. J. E. Gullberg for the photographs; to Mr. Bronislaw Honigberg for maintaining the cultures; to Mr. E. N. Kozloff for making some of the preparations; to Mrs. E. N. Scott for the original specimens of the Berkeley strain used; and to Lt. A. C. Pipkin for the Army Medical School strain. The Cutter Laboratories, Berkeley, generously supplied the horse serum for the cultures, and

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## DESCRIPTION

In suitable Bodian protein-silver preparations, with an intensity dependent on the time and temperature of impregnation, the flagella, costa, undulating membrane border, and axostyle may be shown with great distinctness. On the other hand, the nucleus varies in intensity of impregnation; often it is not blackened, but is gray (Fig. 27) or colorless; still, it is possible unmistakably to observe its size and position. The fact that the nucleus is then not heavily stained aids in observation of the small parabasal body, which lies against its membrane (Figs. 25–27), and could be differentiated only with great difficulty and uncertainty, if at all, in haematoxylin-stained preparations. The blepharoplast-complex is usually not shown, because it is obscured by the relatively large, deeply impregnating structure in the anterior portion of the body.

The general appearance of this deeply impregnating structure is not unlike that of the very large, iron-haematoxylin-stained mass shown anterior to the nucleus in some drawings of *T. hominis* (fig. 46, for example, in the paper by Bishop, 1931); but it is in striking contrast to the small blepharoplast-complex usually represented. It might be thought that the large mass is an accident of impregnation: that is what I thought at first, as it seemed possible that silver technique might be especially likely to give such a result. With further study, however, I became convinced that it is a structure definitively demonstrated, which in the ordinary iron haematoxylin preparations does not appear at all. In the silver preparations it shows in every specimen and is constant in position and shape. The structure will be referred to as the pelta. It is altogether distinct from the blepharoplast-complex.

The pelta is a membrane-like structure which has, in general, somewhat of a crescentic form (Figs. 9, 10, 16, 17). It is situated at the periphery of the body toward the right side in the most anterior part, and lies to the right of the blepharoplast-complex, which may sometimes be seen when impregnation is light as an ill-defined rounded body (Figs. 16, 17). From the point of view of the observer, in the positions in which the trichomonad is most often placed on slides, the pelta lies either over or under the blepharoplasts. Its convex anterior edge is at the extreme anterior edge of the body. The pelta is prolonged in a prominent, tapering extension in its dorsal part (Figs. 4–9). Soon after leaving the main body of the pelta the extension narrows to a filament that is comparable in thickness to the costa. In its first part it is at the periphery of the body; then it turns posteriorly and passes deeper in the cytoplasm dorsal to the nucleus and a short distance to the right of the undulating membrane and costa. There is some variation in its apparent length; in most specimens the length considerably exceeds that of the main body of the pelta. In many specimens the posterior end of the filament is posterior to the nucleus (Figs. 8, 10); in others the filament seems to be shorter (Figs. 4, 6), but it may be that it is not then impregnated for its full length. This dorsal filament extending from the pelta is present in all interphase specimens, and is relatively constant in position and form. It does not come in contact at any place with the nuclear membrane.

At the other end of the pelta (Figs. 2–4, 8, 9, 23) there is a short, narrow extension of silver-impregnating substance along the region ventral to the nucleus; this is probably on the ventral edge of the capitulum of the axostyle. It is likely

that the structure here designated as the pelta is continuous with, or part of, the capitulum. Even so, as a deeply impregnating structure the pelta has an integrity of its own. The flattened capitulum lies on the left side of the nucleus; anteroventrally it is contiguous with the edge of the pelta, which extends on the right side of the blepharoplasts and the plane of the nucleus. The posterior part of the trunk of the axostyle—especially where it projects from the cytosome—often impregnates as deeply as does the pelta (Figs. 21, 27).

In the early prophase stage shown in Fig. 20 the pelta has left its original position, leaving the blepharoplast-complexes visible; and it has been somewhat disorganized. Although it still impregnates deeply it is evidently in process of disintegration. New peltas are probably reorganized in the course of the division process.

I formerly (Kirby, 1943) called attention to the fact that the base of the group of anterior flagella appears in dark-field illumination as a single columnar structure that shows only two edges. The independent flagellum was reported to originate in a bright, clear end entirely separate from, and a micron or so posterior to the base of the group of anterior flagella. This arrangement of the flagella appears in the same manner in the silver preparations. In addition, the pelta appears in the intervening region, and the reason for the arrangement is apparent (Figs. 9, 11, 16).

The blepharoplast-complex is situated on the left side of the pelta and the group of anterior flagella extends anteriorly from it (Figs. 16, 17). In the silver preparations the group is at the base a single columnar structure which appears solid black (Figs. 1, 3, 4, 11, 22). Farther anteriorly the group generally separates into its four component flagella (Fig. 22). The independent flagellum originates from the blepharoplast-complex and passes in an altogether different direction. It emerges under the posterior edge of the pelta, and it extends free on the right side of the body near the ventral border (Figs. 4, 7). Because of the pelta, it is mechanically impossible for the basal part of the independent flagellum to come in contact with the base of the anterior flagella. In living flagellates this flagellum is often directed dorsally on the right side, and moves anteroposteriorly in the vicinity of the undulating membrane.

The two strains that I have studied are characterized by the possession of four anterior flagella and an independent flagellum. The presence of this number of flagella in more than fifty living specimens of the Berkeley strain was reported in 1943. In suitable silver preparations of the Berkeley strain all specimens encountered in a reading of two slides were recorded. On one slide sixty-one specimens had unmistakably four anterior flagella and one independent flagellum. Twenty-nine were recorded as not countable. A few of these were degenerate, but most were not counted because the group of anterior flagella was turned back against the body, and it was not possible to be sure of the number. Also omitted were several specimens in division. In seven specimens counts were made that were not four plus one: two specimens had four anterior flagella but no independent flagellum; two had three anterior and no independent, and three had the independent but apparently only two or three anterior flagella. These seven specimens cannot in any case be interpreted as indicating genetic departure from the complement of flagella typical of fully developed specimens. Some of them were small and

could be post-division stages in which flagella had not all developed. In some the missing flagella may have been overlooked, by reason of position or staining. There might also be abnormal specimens. Some preparations, made from old cultures in which many of the flagellates were more or less moribund, showed great variability in flagella: there was loss of the structures until many had none at all—but obviously, no conclusions can be drawn from the study of degenerate specimens.

On another slide in which almost all of the flagellates were in normal condition, 125 specimens encountered in succession were recorded for flagella. A certain additional number were not recorded because, for the reasons given above, the flagella could not be counted. One hundred and eleven specimens had unmistakably four anterior flagella and an independent flagellum. The fourteen others included some that lacked the independent flagellum, some with apparently only three anterior flagella, and other departures from the normal number. In certain specimens the complement of anterior flagella included three long ones and a very short one, and in some the independent flagellum was very short. Recent beginning of outgrowth was evident in these cases. It is clear that specimens like this in no way suggest the existence of genetic variability in the typical complement of flagella in the flagellates studied.

Studies of flagella of the strain from the Army Medical School, made both by dark-field illumination and silver impregnation, gave similar results. A count of all normal specimens encountered in succession in a reading of one silver preparation gave a record of thirty-four with four anterior flagella and one independent flagellum, and four not countable.

The four anterior flagella are almost always grouped together in their basal portion. In some specimens they have retained this grouping for their whole length, and there appears only a single flagellar column (Fig. 19a). Often in the silver preparations it is possible in such a group to differentiate the ends of the four flagella—which are generally of unequal length. Usually the flagella separate at a short distance from the base. The division may be into 2, 3, or 4 parts (Fig. 19b, c, d; 17), and the thickness of each part is an indication of how many flagella compose it. Groups of two (Fig. 19b, c) or three (Fig. 17) flagella may adhere for all their length; it is evident how this adherence of flagella could mislead an observer of specimens in which they are not very clear and appear very slender. It is reasonably certain that the claim by Duboscq and Grassé (1924) that *Trichomonas trypanoïdes* has 1, 2, 3, or 4 flagella is based on an error of interpretation.

The independent flagellum is normally directed posteriorly, moving in an arc that generally does not carry it forward to parallel the anterior flagella. In many specimens it lies at least in part against the surface of the body; and in the majority it does not appear with the group of four anterior flagella.

The costa is black in some silver preparations, faint in others. The thickness of the costa appears in preparations where the flagella are black, to be about the same as the thickness of an anterior flagellum (Figs. 13, 24). In its posterior part, at the posterior end of the body, the costa tapers to a finer filament (Fig. 18).

The structure of the undulating membrane shows with much distinctness in suitable silver preparations. There are two filaments, one at the edge and the other closely paralleling it; they are separated by a narrow, clear space of constant width (Figs. 4–6). The filaments appear equal to one another in size. Some

specimens are impregnated so heavily that the whole structure—filaments and intervening space—appears as a black band. Near the anterior end the two filaments meet, and a single fine filament passes to the blepharoplast-complex, on the left dorsal side of the pelta. At the posterior end one of the filaments continues in the posterior flagellum, which may appear considerably stouter than the filament. The other filament terminates bluntly near the point where the posterior flagellum becomes free (Fig. 18). The filament that terminates at the end of the membrane is Wenrich's accessory filament. In agreement with Wenrich (1944b) I have observed that the accessory filament seems to be peripheral to the other one.

Often some granules are present in the region between the costa and the outer edge of the membrane; generally these are closer to the costa. Frequently, at least in places, the granules are arranged in an incomplete row. They are the paracostal granules described by Wenrich. They vary in size, and they often impregnate deeply with protargol.

The silver preparations show further features of interest at the ends of the flagella. In regard to the terminal differentiation there are two types of flagella in this flagellate: the posterior flagellum constitutes one type and the other five flagella the other type. Generally, the posterior flagellum ends in a filament (Fig. 15) that is much more slender than the rest of the flagellum and has a very variable length of up to about 5 μ. Sometimes this filament appears smooth and continuous, but more often it appears uneven and broken. The existence of this differentiation is indicated in Wenrich's account (1944a, p. 194). I am unable to say, however, whether the filament is a free axoneme or not.

The ends of the anterior flagella (Figs. 14, 21) are usually different in appearance from that of the posterior flagellum. There is no prolongation in a filament, and the end is blunt. Occasionally, the terminal part appears to be attached by a filament to the rest of the flagellum, but that is not the usual form. Sometimes the end is not distinguished from the rest in any way. Often, however, it may be seen in fixed specimens that the terminal part, up to half a micron or so in length, is bent to one side, generally slightly, but sometimes as much as a right angle. (This feature is indicated in one of the flagella in Wenrich's Fig. 4, 1944a.) This terminal structure impregnates black, and may be sharply set off in intensity of staining from the part posterior to it. It varies in length. Sometimes it appears like a black granule or a slight enlargement at the end of the flagellum. In most specimens it appears as a black rod, straight, bent, sometimes somewhat curved. It may suggest a claw or the head of a golf club. The terminal differentiation resembles in size and shape some of the bacilli that are present in very diverse size and form in this culture. But its consistent occurrence at the end of the flagella leaves no doubt that it is actually part of the flagellar structure.

Dark-field illumination of the flagellate shows the exterior structure with much distinctness. The free flagella are very substantial, solidly illuminated strands with an apparent thickness comparable to that seen in well-impregnated silver preparations. The filament at the end of the posterior flagellum can be found in many specimens, but others do not show it. With a few exceptions, no filament appears at the end of the anterior flagella, which generally terminate bluntly, in ends that are as thick as any other part. Often the terminal section, corresponding to the part that is especially deeply impregnated in protein-silver, seems especially bright

and somewhat thicker than the rest. It was sometimes seen to be bent at an angle to the rest of the flagellum, but that feature was noted much less frequently in the dark-field preparations than in the fixed specimens.

The undulating membrane margin shows in dark-field two parallel filaments, each finer than a free flagellum. This structure can be made out only in specimens in which the membrane is moving slowly, not after death of the flagellate.

The projecting part of the axostyle appears as a rod with two parallel bright edges. The projection from the cytoplasm is moderately long; and there is no taper until near the posterior end, when the rod is rather abruptly sharpened. It terminates in a point, usually without prolongation in a filament. The interior of the rod is optically empty, except for a few small granules in some instances. In the movements of the specimens observed the projecting axostyle was a rigid rod, and did not bend even passively. One specimen was dragging a dead trichomonad and a group of bacteria at the end of a rather long filament prolonged from the end of the axostyle. Even when the axostyle was jerked laterally against the resistance of this attached mass it did not bend. Eventually, the dead trichomonad became lodged between masses of débris and pulled away, leaving the axostyle of the active flagellate sharpened but without a filament.

Demonstration of the parabasal body is a particularly interesting result of the use of Bodian's protein-silver method. This structure has not been seen before in *Pentatrichomonas hominis*. Wenrich observed in some iron-haematoxylin-stained specimens a fine fibril in the position normally occupied by the parabasal body in trichomonads. He could not suggest, however, whether the structure was a parabasal fibril or a new costa. There is also the possibility that it might have been a part of the dorsal filament extending from the pelta, but the position is not the same, since Wenrich's fibril is entirely between the nucleus and the costa. The structure that I consider to be the parabasal body is deeply impregnating, small, ellipsoidal body of small size against the anterolateral surface of the nucleus (Figs. 4–12, 25–27). It is not between the costa and the median longitudinal plane of the nucleus, but lies a short distance to the right of the plane passing through those two structures. In some specimens the parabasal substance is divided into two granules (Fig. 17). Sometimes a filament (Fig. 4) can be traced from the anterior end of the parabasal until it passes under the pelta, doubtless to meet the blepharoplast. No filament was observed passing posteriorly. Protargol might not be expected to demonstrate the filament well, in the light of the reaction of the parabasal apparatus of some other TRICHOMONADIDAE to the technique. It is possible that there exists a filament comparable in its extension beyond the parabasal substance to that of *Trichomonas tenax* and *T. vaginalis;* and since Wenrich's filament is in just the position it would be expected to occupy, it may be that that is the structure.

## DISCUSSION

From a taxonomic point of view, the most significant characteristic of the trichomonad of which an account is given in this paper is the existence of the independent posteriorly-directed flagellum. It has been observed in this and related flagellates by various authors (Kofoid and Swezy, 1923; Tanabe, 1926, 1940; Wenrich, 1944a, b; and others), and it has been known that it originates from a separate blepharoplast. Most writers have not differentiated it clearly from the group of

anterior flagella, and have written as though the five-flagellated trichomonads simply have one anterior flagellum more than the typical *Trichomonas,* as the three-flagellated species have one less. Tanabe (1940) noted that in *Pentatrichomonas felis,* which is identical with *T. hominis* according to various authors (Wenrich, 1944a), this flagellum trails immediately after emergence. Actually, the independent flagellum does not in any sense belong with the group of four anterior flagella. It is more nearly to be compared with the trailing flagellum of *Peranema* or *Eutreptia* in its position in relation to the body. It should, therefore, be recognized as a new feature of structural organization added to that of *Trichomonas.* The internal structures of the mastigont and the undulating membrane show considerable differences among trichomonads in degree of development, but I do not think that those differences are so significant for group differentiation as is this independent flagellum. It seems to me that the presence of that structure warrants generic differentiation of the intestinal trichomonad of man; and I think that the taxonomic differentiation should be recognized by assigning to the flagellate the name *Pentatrichomonas hominis* (Davaine, 1860). Wenrich, 1931, seems to have been the first to compose the name in this combination.

If, as is likely, the pelta is a development of the anterior part of the capitulum of the axostyle, a comparison may be made with the extensions of the capitulum that occur in certain DEVESCOVININAE, the highly differentiated group of TRICHOMONADIDAE in termites. The pelta is demonstrated very clearly by use of silver technique; but earlier studies, which did not include that technique, have not recorded it at all. Very little is known about the structure of flagellates as revealed by silver impregnation, and undoubtedly there is much to be learned by application of this valuable technique.

The structural differentiation of the terminal parts of the flagella is another feature whose study will be furthered by use of silver technique. Attention has been directed to structure of the flagella of many flagellates (Vlk, 1938). One type is the Peitschengeissel, so named because of the existence of a fine terminal filament. All of the flagella on *Bodo, Trepomonas,* and *Hexamitus* studied by Vlk were found to be of this type. It is also the type of the posterior flagellum of *Pentatrichomonas hominis.* The anterior flagella have a different structure, a substance at the ends that impregnates more deeply than the rest, and in fixed specimens may be set at an angle to the rest of the flagellum.

The very small parabasal body of *Pentatrichomonas hominis* has not hitherto been reported. Silver preparations may show the parabasal substance well, but fail to demonstrate the filament; that has been noted in various trichomonadin and devescovinin flagellates. The account of the parabasal apparatus given in this paper must, then, be regarded as not necessarily complete. Further search for a filament, which may as in *Trichomonas tenax* extend far posteriorly to the parabasal substance, must be made. It is possible that Wenrich (1944a) did see the filament, but for certainty it is necessary that both substances of the parabasal apparatus should be revealed at the same time.

The genus *Pentatrichomonas* contains intestinal flagellates of various vertebrates. Wenrich (1944a) suggested that the forms in man, monkeys, cats, dogs, and rats all belong to the one species. There is also *Pentatrichomonas macropi* Tanabe, 1926, of kangaroos; *P. eutamias* Tanabe and Okinami, 1940 of *Eutamias asiaticus;*

and perhaps the five-flagellated trichomonad from poultry, as described by Allen (1936, 1940). In *P. macropi* from "the kangaroo" the independent flagellum shown by Tanabe is comparable in length to the anterior flagella; but in *P. macropi* from four species of *Macropus* and one of *Dendrolagus* the length of the independent flagellum shown by Herman (1939) is more than twice that of the anterior flagella —a relative length that is never reached in the strains of *P. hominis* that I have studied. Because silver preparations make possible the study of additional structural detail, they would be valuable for comparison. Differences in the pelta and the parabasal body would be highly significant. Until comparisons are made with regard to those, as well as other features, it is not possible to state that all these forms are morphologically identical with one another. The flagellate which in 1931 I described under the name *Pentatrichomonoides scroa*—a trichomonad that occurs in various termites—was mistakenly listed by Kudo (1939) as *Pentatrichomonas*. Its organization is very different from that of *Pentatrichomonas hominis*. The generic name is not very suitable, since it has resulted in confusion and suggests a closer similarity than that which exists.

SUMMARY

In Bodian protein-silver preparations many features of the mastigont structure of an intestinal trichomonad of man can be demonstrated with much distinctness. Some of these features have not hitherto been reported. At the anterior end of the body is a flattened structure, the pelta, which lies to the right of the blepharoplast-complex and is prolonged dorsally in a filament that passes posteriorly in the cytoplasm dorsal to the nucleus. The pelta may be an extension of the capitulum of the axostyle. The margin of the undulating membrane consists of two parallel filaments, as Wenrich reported; one of these continues in the posterior flagellum, which generally terminates in a slender filament. The other flagella are typically as stout at the ends as elsewhere; and have a terminal section that impregnates especially deeply with silver, and sometimes is bent at an angle. A small, ellipsoidal parabasal body lies against the anterolateral surface of the nucleus, a short distance to the right of the plane passing through the nucleus and costa. The parabasal apparatus may also include a filament, like that in *Trichomonas tenax,* but it was not demonstrated in this material. This flagellate from man possesses a normally constant number of four anterior flagella in a group, an independent flagellum, and the undulating membrane with its posterior flagellum. The independent flagellum is directed posteriorly, arises under the posterior margin of the pelta, and is entirely separate from the anteriorly-directed group of four flagella. It is believed that this feature justifies generic distinction of this flagellate of man, as well as of similar forms in other animals. The intestinal trichomonad of man may be denominated *Pentatrichomonas hominis* (Davaine, 1860).

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## EXPLANATION OF PLATES

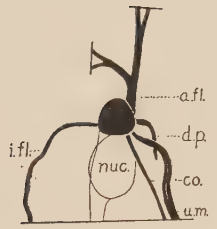
All figures are of *Pentatrichomonas hominis* (Davaine)

Abbreviations

a. fl.—anterior flagella
ax.—axostyle
co.—costa
bl.—blepharoplast-complex
d. p.—dorsal filament extending from pelta
i. fl.—independent flagellum
nuc.—nucleus
pe.—pelta
p. b.—parabasal body
p. fl.—posterior flagellum
u. m.—undulating membrane
v. p.—ventral extension of pelta

### PLATE 1

FIGS. 1–12. Camera lucida drawings of the mastigont structures of the anterior part of the body. In all specimens the anterior flagella originate in a columnar structure which later divides into four flagella. Where there appear to be two or three, actually some are united with others. The margin of the undulating membrane consists of two parallel filaments. The pelta is extended dorsally in a filament that extends deep in the cytoplasm near the costa. There is a ventral extension along the ventral edge of the capitulum of the axostyle. The parabasal body is against the nuclear membrane near the costa. In a view from the dorsal side (Fig. 12), it shows alongside the nucleus. In the other figures it lies either above or below the nucleus, but focusing indicates that it is outside the membrane. Hollande cupric picroformal. Bodian protein-silver. ×3500.

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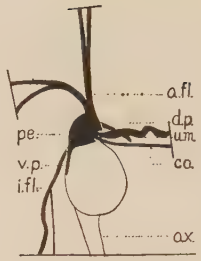


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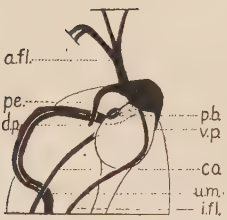


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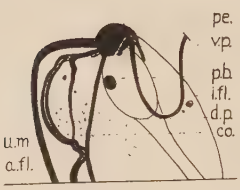


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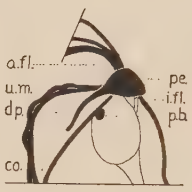


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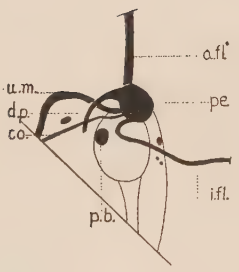


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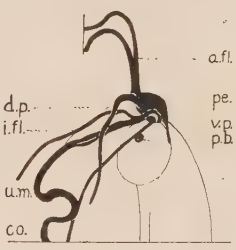


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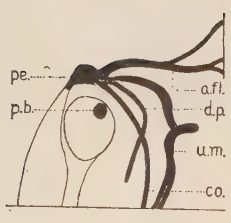


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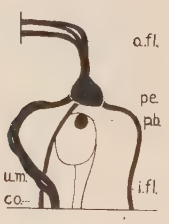


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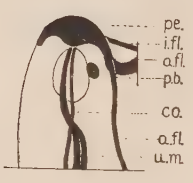


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PLATE 1

## Plate 2

Fig. 13. *Pentatrichomonas hominis* entire. The margin of the undulating membrane consists of two parallel filaments. The dorsal filament extending from the pelta, most of which lies between the nucleus and costa in this specimen, appears also in Fig. 24, which is a photograph of the same specimen. ×2400.

Fig. 14. Distal parts of the anterior flagella, showing the terminal differentiation that impregnates more deeply than the rest with protein-silver. The terminal differentiation shows in the photographs Figs. 21 and 22. Freehand diagrams.

Fig. 15. Terminal filaments of the posterior flagella, sometimes absent. Freehand diagrams.

Fig. 16. Portion of the mastigont of a specimen in which the pelta is not very heavily impregnated, and the blepharoplast-complex can be seen as a separate body, giving origin to the mastigont structures. ×3500.

Fig. 17. Pelta and blepharoplast-complex can both be seen; three anterior flagella adherent in one group, one partly free. Parabasal body appears as two granules. ×3500.

Fig. 18. Diagram of the posterior end of a mastigont, margin of the undulating membrane partly pulled away from the edge of the cytosome, which is marked by the costa. One of the marginal membrane filaments continues in the posterior flagellum; the other terminates at the end of the membrane.

Fig. 19. Diagrams to show various types of groupings of anterior flagella. The pelta is also shown. An independent flagellum is present in each specimen, but is not shown in the drawing. In *a* the four flagella are united, each has a different length, and the end of each can be seen along the column. In *b* the flagella are united in pairs. In *c* two flagella are separate, two united but of different length and the ends can be seen. In *d* the four flagella are separate except at the base.

Fig. 20. An early prophase. There are two blepharoplast-complexes, represented here somewhat diagrammatically, which give rise to sets of mastigont structures. There is a parabasal body associated with each mastigont. The original pelta is displaced, is in disorganization, and new ones have not begun to develop. ×3500.

## Plate 3

Photographs of *Pentatrichomonas hominis,* made by J. E. Gullberg, not retouched. Specimens fixed in Hollande's fluid, impregnated by Bodian's protein-silver technique.

Fig. 21. The independent flagellum is turned forward in a position that is not the usual one. Terminal differentiations show at the ends of the anterior flagella, and a filament on the posterior flagellum.

Fig. 22. This and the preceding figure show the origin of the four anterior flagella in one group, and the separateness from this of the independent flagellum.

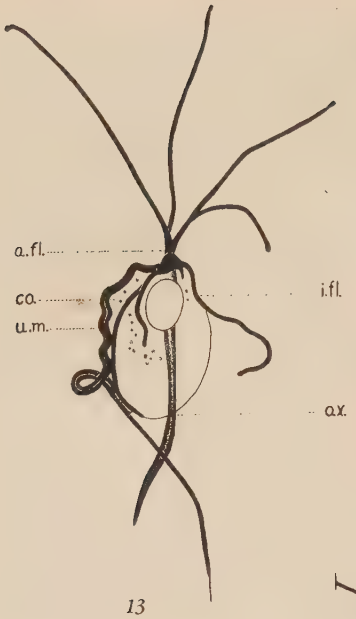
Fig. 23. Shows the pelta, part of the dorsal filament that extends from it, the ventral extension of the pelta, a group of four anterior flagella, and the independent flagellum turned forward.

Fig. 24. Specimen shown also by drawing Fig. 13. Dorsal to the nucleus is the dorsal filament from the pelta.

Fig. 25. The parabasal body appears as a large rounded granule posterior to the pelta. The dorsal filament from the pelta can also be seen.

Fig. 26. Different focus on the same specimen shown in Fig. 23. Parabasal body applied anterolaterally to the surface of the nucleus.

Fig. 27. Parabasal body posterior to the pelta; near the costa a cytoplasmic granule similar in size. Two of the group of four anterior flagella are adherent until near their ends. The independent flagellum is on the ventral side of the body parallel to the posterior flagellum.

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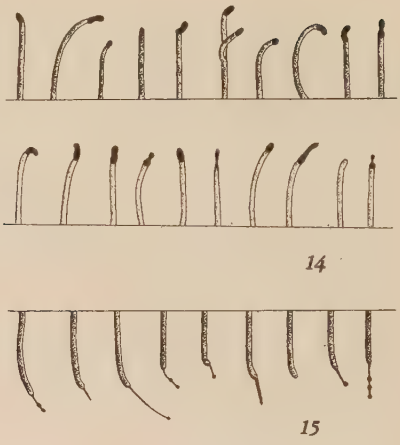

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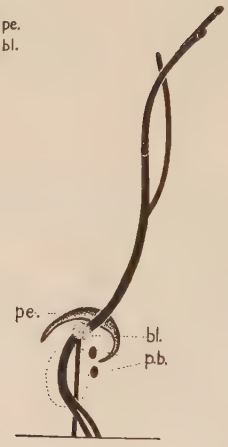


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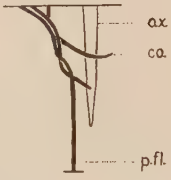


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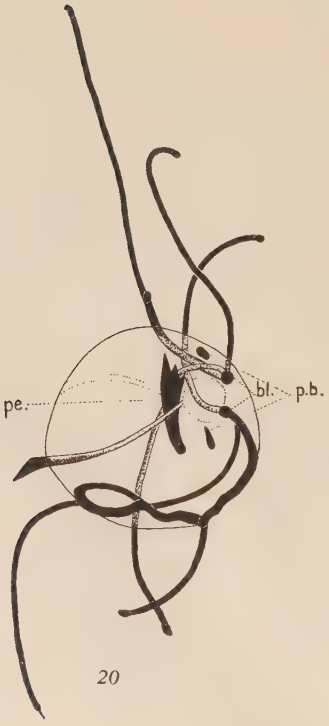


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PLATE 2

PLATE 3

# *ENTAMOEBA COLI* VERSUS *ENDAMOEBA COLI*

HAROLD KIRBY

University of California, Berkeley

In drawing up the argument for Opinion 99 of the International Commission on Zoological Nomenclature, Stiles showed a wish to reject the name *Entamoeba* in the interest of clear distinction. He wrote: "it seems obvious that unless the name *Entamoeba* is definitely suppressed both the nomenclatorial and the taxonomic status of the species which come into consideration will become even more confused." The result of his reasoning was rejection of the name, but the benefits that he hoped for have not been realized. There have been some who in following the Opinion have been influenced to take a position regarding the taxonomic status of the amoebae that is not in accord with clear distinction, because unless they took that position the necessary large nomenclatorial departure from the usage that is very widespread in the literature of medical zoology would indeed result in confusion. Retention of the two names *Endamoeba* and *Entamoeba* would permit a clear-cut taxonomic differentiation to be made at the same time that a minimum of departure from customary usage is necessitated. Therefore it seems to me that Opinion 99 has actually increased the difficulty that Stiles wished to avoid. I agree with Dobell (1938) that the Opinion in its present form should not be regarded as decisive.

The equivocal interpretation that some authors have made of Opinion 99 is illustrated by Craig's criticism (1944) of Kudo's retention of the name *Entamoeba:* "It would have been much better had he followed the ruling of the International Committee of Zoological Nomenclature and used the spelling recommended by it as preferable, i.e., 'Endamoeba.'" Kudo took the position that the species *coli* should not be put into the same genus as the species *blattae,* and his failure to follow Opinion 99 made it possible for him to choose *Entamoeba* as the generic name for *coli.* It is not a question of alternative spelling of the name of the genus: the Opinion does not constitute an approval of the spelling *Endamoeba* as against *Entamoeba.* There is no choice of orthography: *Endamoeba* is correct and has priority as the name of the genus typified by *E. blattae;* all amoebae in that genus are called *Endamoeba,* and those not in that genus cannot be called *Endamoeba.*

The Opinion was published in 1928, and so far as I know, between that time and this only two names have been used in connection with the species *coli, histolytica* and *gingivalis: Endamoeba* by those who put the three amoebae into the same genus as *blattae,* and *Entamoeba* by those who do not. The authors in the former group do not accept the generic distinction; whether or not they follow Opinion 99 does not properly enter into their adoption of *Endamoeba.* The authors in the latter group do recognize the generic distinction, and do not follow the

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[1] Mr. Francis Hemming has, at my request, worded a footnote for this paper as follows: As the subject discussed in the present paper relates to the possible revision of Opinion 99 of the International Commission on Zoological Nomenclature, this paper has been communicated also to the International Commission for the information of the Commissioners. In order that the International Commission may be in a position to inform itself of the views of all interested specialists, all comments on the issues raised in the present paper should be sent direct to the Secretary of the International Commission at the Commission's Publication Office, 41 Queen's Gate, London, S. W. 7.—H. K.

Opinion. If there should be a third group of authors, who recognize generic distinction among these endozoic amoebae, and accept Opinion 99, it would be necessary for them to write *Poneramoeba histolytica, Poneramoeba coli,* and *Poneramoeba gingivalis.*

The following chronological summary of the history of this matter sets forth the important facts that need to be considered:

1879. Leidy (1879a, p. 300) introduced the generic name *Endamoeba,* with the one species *Endamoeba blattae,* named *Amoeba blattae* by Bütschli in 1878. The same proposal was printed on December 2 in Leidy, 1879b, p. 205.

1895. Casagrandi and Barbagallo introduced the generic name *Entamoeba,* giving as the included species *Amoeba coli* (Lösch) and *Amoeba blattarum* (Bütschli). They were ignorant of Leidy's name.

1897. Casagrandi and Barbagallo (p. 163) again printed the name *Entamoeba,* giving as the included species *Entamoeba hominis* and *Entamoeba blattarum.*

1903. Schaudinn, using the generic name *Entamoeba* C. & B., divided *Amoeba coli* of Lösch into two species, *Entamoeba coli* Lösch and *Entamoeba histolytica* n. sp. He did not mention the species *blattae,* and probably was ignorant of Leidy's name.

1910. Chatton assigned various endozoic amoeba to the genus *"Entamoeba* Leidy (1879)." Among the included species were: *Entamoeba coli* (Lösch) 1875, emend. Schaudinn (1903); *E. blattae* (Bütschli) 1878; *E. ranarum* (Grassi) 1881; *E. histolytica* Schaudinn 1903. Chatton stated that the paternity of *Entamoeba* had been wrongly attributed by authors to Casagrandi and Barbagallo. He made no reference to the fact that Leidy's name was actually *Endamoeba.*

1912. Séance du 14 février, mémoire paru le 5 mars (acc. to Chatton, 1912). Chatton and Lalung-Bonnaire wrote (p. 142): "La dénomination non latine d'Entamibes, appliquée aux amibes normalement parasites du tube digestif est d'un usage commode qui la fera conserver. Mais traduite en nom générique latin, elle ne peut plus s'appliquer actuellement aux amibes du tube digestif des Vertébrés. Ce n'est pas, en effect, à ces dernières qu'elle a été appliquée en premier lieu. C'est Leidy qui a créé le genre *Entamoeba* pour l'amibe de la Blatte, et ce n'est qu'en 1897 que Casagrandi et Barbagallo l'ont appliquée aux amibes intestinales des Vertébrés." The authors considered that the amoebae of vertebrates must be put in a separate genus, for which they proposed the name *Löschia,* to contain *coli* Lösch and other species.

1912. Séance du 26 mars. Chatton reported again the generic differentiation of endozoic amoebae made in the above paper. He definitely designated *Löschia coli* Lösch, cysts 8 nuclei or more, as type of the genus *Löschia.* Remarking that protistologists had wrongly attributed the paternity of the genus *Entamoeba* to Casagrandi and Barbagallo 1897, he wrote (p. 111): "Ces derniers avaient bien appliqué le nom d'*Entamoeba* à une Entamibe humaine, mais Leidy l'avait donné des 1879 a l'*Amoeba blattae* de Bütschli." In a footnote to this statement he noted that Leidy's spelling was *"Endamoeba,"* but dismissed that name as an orthographic variant.

1913. Brumpt wrote of the amoebae of man under the name *"Entamoeba* Leidy, 1879," making the same mistake for *Endamoeba* that Chatton as well as Alexeieff (1912) had previously made. In a footnote (p. 21) he wrote: "Ce même genre a

été créé de nouveau en 1897 par Casagrandi et Barbagallo pour leur *E. hominis,* synonyme de *E. coli.*" That sentence has been accepted by Stiles and Boeck (1923, p. 122), Stiles and Hassall (1925, p. 8), and Stiles (1928 in Opinion 99) as a designation of the type *E. coli* (as *E. hominis*) for *Entamoeba* Casagrandi & Barbagallo. (In the third edition, 1922, Brumpt made the same error of "*Entamoeba* Leidy"; but in the next one, 1927, he used *Entamoeba* C. & B. and noted that *Endamoeba* Leidy should be reserved, in agreement with Wenyon, 1926, for the amoeba of the roach.)

1919. Dobell used *Endamoeba* Leidy, 1879, for *E. blattae* only, and *Entamoeba* Casagrandi & Barbagallo, 1895, for *E. coli, E. histolytica,* and *E. gingivalis.*

1923. Stiles and Boeck, in a study of the nomenclatorial status of the protozoa of man (p. 125), considered the genus *Endamoeba* Leidy, 1879, with two subgenera: *Endamoeba* Leidy, 1879 (type by monotypy *E. blattae*) and *Poneramoeba* Luehe, 1909 (type by monotypy and original designation *E. histolytica*). They stated (p. 124) that "*Entamoeba* 1895 is not available because of *Endamoeba* 1879"; evidently that is because they thought of *Entamoeba* as a homonym, or orthographic variant, because here they dealt with a separate taxonomic category (the subgenus) from *Endamoeba.* The type of *Entamoeba* 1895 is given (p. 122, 124) as *E. hominis* tsd.= *coli* and *coli* (s. *hominis*); type by subsequent designation is by Brumpt, 1913.

1925. Stiles and Hassall, in the "Key-Catalogue of the Protozoa Reported for Man," list (p. 8) *Entamoeba* C. & B., 1895, type by subsequent designation *hominis* = *coli,* as a subjective synonym of *Endamoeba* Leidy, 1879. It appears from the definition of subjective synonym by Stiles and Boeck, 1923, that it is a category providing for cases where the identity in question is not absolute, but depends on "the experience and judgment of the reviser" (p. 138). Since in the key-catalogue the types of *Entamoeba* and *Endamoeba* are given as different, although those two types are treated as members of the same genus, it is likely that the reference is to the difference of opinion about generic assignment. Otherwise *Entamoeba* would simply have been designated as a homonym; that category is dealt with in the same paper. Reference to *Entamoeba* as a synonym is, therefore, evidently on the basis that its type, *Ent. coli,* belongs in the same genus as *End. blattae,* according to Stiles and Hassall.

1928. The International Commission on Zoological Nomenclature published Opinion 99 in response to an inquiry as to whether the names *Endamoeba* and *Entamoeba* should be considered homonyms. The summary of the Opinion reads: "*Entamoeba* 1895, with *blattae* as type by subsequent (1912) designation, is absolute synonym of *Endamoeba* Leidy, 1879a, p. 300, type *blattae,* and invalidates *Entamoeba* 1895, type by subsequent (1913) designation *hominis* = *coli.*" The report also contained the decision that *Entamoeba* is a homonym of ("philologically the same as") *Endamoeba.* It is presumably on that basis that the Secretary recommended that "the name *Entamoeba* 1895, either with type *hominis* = *coli* as definitely designated by Brumpt, 1913, p. 21, or with *blattae* as accepted by Chatton and Lalung (1912, 111) and as implied by Chatton (1910, 282), be definitely invalidated by *Endamoeba* Leidy, 1879a, p. 300, type *blattae,* irrespective of the point whether the type of *Entamoeba* be considered *blattae* or *coli.*" (The reference to Chatton and Lalung, 1912, p. 111, is evidently a mistake for Chatton and Lalung-Bonnaire, 1912, p. 142, or for Chatton, 1912, p. 111.)

It is evident from this summary that Stiles (1928) was justified in his statement that "the case has already produced considerable confusion in literature." It is also evident, however, that this confusion need not have existed if authors had simply been attentive to the correct forms. Then *Endamoeba* Leidy would have been used for any generic concept including the species *blattae;* and *Entamoeba* C. & B. would either have been rejected, or used solely for any generic concept omitting *blattae* and including *coli.* The errors made by earlier authors should not influence us in an effort to reach a solution of the problem.

The answer to the taxonomic problem is subject to differences of opinion. Many authors follow the usage of Stiles and Boeck, 1923, in writing *Endamoeba coli;* that usage has been almost universal in American compilations in medical zoology since it was adopted in 1926 by Craig (who in 1911 used *Entamoeba* Casagrandi and Barbagallo without reference to the genus *Endamoeba* Leidy). American writers who recognize generic distinction between *blattae* and *coli* include Kudo (1939, 1944), Wenrich (*Entamoeba* used for *histolytica* and *coli* in 1940, 1944, and other papers of similar date), Cleveland (Cleveland and Sanders, 1930, and other papers), Pearse (1942), and Meglitsch (1940, in connection with a profound study of *blattae*). And there are many in various parts of the world who follow the same course; (for example, Wenyon, 1926; Dobell, 1919, 1938; Brumpt, 1936; Reichenow, 1928), so that it is not a question of individual or even minority disagreement in the question of taxonomic differentiation.

It is not my purpose in this paper to attempt to defend one position in taxonomy or attack the other. Because of the very large difference between the species *blattae* and *coli* in the nuclear structure of the trophic forms, I think that the burden of proof should rest on those who assert that the two amoebae belong in the same genus—especially when the same authors recognize as valid certain other genera of endozoic amoebae. A comprehensive analysis of the taxonomy of all amoebae, free living and endozoic, is much to be desired. Morris (1936) examined the problem so far as certain endozoic amoebae are concerned; but the result of his study is not conclusive, for it omitted from consideration certain other endozoic amoebae that would also have to have the status of subgenera of *Endamoeba,* according to his treatment. The purpose of the present paper is nomenclatorial: it is an attempt to show that the word *Entamoeba* should remain available for a genus of which *Ent. coli* is the type.

Opinion 99 declares that those of us who think that the species *coli* and similar forms do not belong in the same genus as *blattae* cannot use the name *Entamoeba* for that different genus. There are two grounds upon which that declaration is based. One of them is that *Entamoeba* is a homonym of *Endamoeba*—that the two words are not sufficiently different from one another in orthography to be usable as separate words. The other is that *Entamoeba* has the same type species as *Endamoeba,* and therefore falls as a synonym. The latter decision is the only one given in the summary of Opinion 99; it is not necessary that it should be rendered after the generic name has been dismissed as a homonym, so evidently it is intended to provide a reserve in case of doubt.

That doubt certainly exists (Dobell, 1938). Obviously we are not here concerned with whether the words have the same meaning or not, but with whether one word is the same word as the other. There is a difference between inadvertent

interchange of two names that have a status in zoological nomenclature, and the use synonymously of such words as endoplasm and entoplasm or endoderm and entoderm. There is nothing in the articles of the International Rules of Zoological Nomenclature that justifies the conclusion that *Entamoeba* must be rejected as a homonym. Certainly Chatton's statement, although cited as authoritative by Stiles, does not constitute justification; it is merely an assertion in a one line footnote, unsupported by reference to the Rules or anything else. It is only in the argument for Opinion 99 that evidence is given, but that evidence can as well be read in support of the retention of the two names as different. Jordan's report in the Opinion states that they come in the category of names of which the spelling in Latin varied to a slight extent and which the Rules of Nomenclature do not accept as different. His reference is to Article 35, in which precise differences are given by which specific names of the same origin and meaning are insufficiently distinguished. There seems to be no indication that Article 35 is intended to establish a general category allowing interpretation of other differences than those specified; and in that Article there is nothing whatever about the sort of difference that exists between the words *Endamoeba* and *Entamoeba*. Furthermore, there is evidence in Opinion 99 itself that the two words are not necessarily of the same origin, and that would exclude them from consideration under the rules given in Article 35.

Article 35 deals only with specific names, and it might seem possible that a different interpretation for generic names would be allowed. Now, however, a precise statement concerning differences in generic names has been given (Opinion 147, 1943). A generic name of the same origin and meaning as a previously published generic name is to be rejected as a homonym of the said name if it is distinguished therefrom only by certain specified differences, which are the same ones given for specific names in Article 35. Opinion 99 was not mentioned by the Commission in the rendering of Opinion 147, although it dealt with the subject that was being considered.

It is not possible to find any definite reason in the Code, or any valid evidence in Opinion 99, for rejection of *Entamoeba* as a homonym; but the recommendation in Article 36 can, as Taliaferro remarked, be evoked in support of retention of both names. These facts have already been discussed by Dobell (1938).

In Opinion 99 the consideration that is apparently regarded as the more important one, since it alone is given in the summary, is that of synonymy—that *Entamoeba* C. & B. is an absolute synonym (or objective synonym, Stiles and Boeck, 1923, p. 135) of *Endamoeba* Leidy, because the names follow their types, and the same species, *E. blattae,* is the type of each. When Stiles presented the case so as to arrive at this conclusion, he changed his approach to the matter. In 1923 he evidently regarded *Entamoeba* as a homonym, in 1925 he designated it as a subjective synonym on the basis of the taxonomic assignment of its species, but in both papers he accepted *E. hominis* = *coli* as type of *Entamoeba* C. & B. by subsequent designation by Brumpt, 1913. In Opinion 99, after stating that Brumpt's action was the first type designation in words, Stiles found it possible to interpret Chatton, 1912, as having designated *blattae* as type of *Entamoeba* C. & B. Stiles did not make clear the reason for this interpretation, except in that he cited Opinion 6 in support of it.

*Entamoeba* C. & B., 1895, is analogous to the hypothetical Genus *A* Linnaeus, 1758, in Opinion 6, in that when proposed it contained two species, which we now know as *coli* and *blattae*. Casagrandi and Barbagallo did not indicate which was the type. Opinion 6 declares that when an author has removed one of the two species to another monotypic genus, leaving only one species in the first genus, he is to be construed as having fixed the type of the first genus. Jordan's report in Opinion 99 follows the parallel exactly, crediting Chatton with having removed *coli* from *Entamoeba* C. & B. to the genus *Löschia,* thereby leaving *blattae* as the type of *Entamoeba.* If that is so, there is probably no doubt about the validity of the conclusion; but I think it is not true that Chatton really dealt with *Entamoeba* C. & B. in making the supposed division.

In every place in the three papers that Chatton wrote *Entamoeba* Leidy he was simply making a mistake for *Endamoeba* Leidy. Other authors before him who included *blattae,* with or without other amoebae, in *Entamoeba* C. & B. were also making a simple error; they should have used *Endamoeba* Leidy. The acts of Chatton and Lalung-Bonnaire were on *Endamoeba* Leidy, given by mistake as *Entamoeba* Leidy, but not corresponding to *Entamoeba* C. & B. Chatton (1910) grouped various amoebae in this *"Entamoeba* Leidy." Chatton and Lalung-Bonnaire (1912) did not agree with that grouping, and removed *coli* and other species from it, leaving only *blattae.* That made no change in the situation, except to restore it as it was originally. The revision was of the group concept authors had held of *"Entamoeba* Leidy" = *Endamoeba* Leidy, not of that genus itself, which was already attached to its type species.

The synonym argument in Opinion 99 depends upon crediting Chatton and Lalung-Bonnaire or Chatton with having comprehended *Entamoeba* C. & B. in what they did with *"Entamoeba* Leidy" = *Endamoeba* Leidy. Stiles' paragraph "d" in the argument, puts it: "Chatton's paper (1912, Bull. Soc. zool. France, p. 113) is to be interpreted as designating *blattae* as type of *"Entamoeba"* 1897 (= 1895) [emendation of *Endamoeba,* but obviously construed as identical with *Entamoeba*]." (Chatton and Lalung-Bonnaire had priority in this matter, and the page reference is wrong.) But Chatton's "emendation of" (rather, error for) *Endamoeba* was *"Entamoeba* Leidy," not *"Entamoeba* 1897 (= 1895)"; *Entamoeba* C. & B., 1895 was not an emendation, but a separately proposed word. Stiles' word "obviously" could have reference only to Chatton's opinion (1912) that the two words are orthographic variants, and therefore identical. Thus we return to the question of whether or not it is to be admitted that *Entamoeba* is a homonym of *Endamoeba;* and in consequence it appears to me that the whole argument of Opinion 99 stands or falls with the decision about the homonym question, in spite of the fact that the summary neglects that decision.

The summary of Opinion 99 presents the nomenclatorial treatment accorded *Entamoeba* C. & B. by Brumpt in 1913 as opposed to and invalidated by the prior treatment of that genus in the 1912 papers. On the contrary, however, it seems that Chatton and Brumpt had then exactly the same understanding of Casagrandi and Barbagallo's genus. In the historical account given above I quoted the statement by Chatton and Lalung-Bonnaire (1912, p. 142) that in 1897 Casagrandi and Barbagallo applied the name *Entamoeba* to intestinal amoebae of vertebrates, and the statement by Chatton (1912, p. 111) that those authors applied the name to a

human amoeba. Those are the only references in the 1912 papers to the correct and original use of *Entamoeba.* Brumpt, who in 1913 wrote *"Entamoeba* Leidy," had adopted the nomenclature of amoebae used by Chatton in 1910. In the footnote that was accepted by Stiles as constituting the type designation he simply gave a different wording of what the 1912 authors had pointed out regarding the amoeba for which the genus *Entamoeba* C. & B. had been proposed; but in that wording, and in printing the name *"E. hominis* synonyme de *E. coli"* he was more specific. Brumpt has more recently used both *Endamoeba* and *Entamoeba;* and it is likely that the 1912 authors would have used Casagrandi and Barbagallo's name for the species *coli* and other amoebae of vertebrates instead of *Löschia* except for the fact that they considered *Endamoeba* and *Entamoeba* to be orthographic variants. Despite the fact that Chatton and Brumpt evidently had the same understanding of *Entamoeba* C. & B., Stiles found it possible to give the interpretation that Chatton had designated *blattae* as its type before Brumpt designated *coli* as its type. Yet the only difference in the treatment the two authors gave the genus is that the former did not print the species name, whereas the latter did so. Brumpt, therefore, was not considered to have comprehended *Entamoeba* C. & B. in *"Entamoeba* Leidy," as regards type designation, whereas Chatton was considered to have done so. The interpretation given in this part of the argument in Opinion 99 is obviously greatly strained.

## CONCLUSION

Opinion 99 of the International Commission on Zoological Nomenclature does not constitute proof that *Entamoeba* Casagrandi & Barbagallo, 1895 cannot be used as a generic name. Its argument rests on two points: that *Entamoeba* is a homonym of *Endamoeba;* and that *blattae* is the type species of both, so that *Entamoeba* falls also as a synonym of *Endamoeba* Leidy, 1879. The latter point, which is the only one brought out in the summary of Opinion 99, is not acceptable: it rests on the interpretation that *Entamoeba* is a homonym of the earlier name. The Opinion asserts, but does not demonstrate, that it is a homonym; and there is nothing elsewhere in the Rules or Opinions that warrants the assertion. It is appropriate to place the species *coli* and *blattae* in separate genera; and it is considered that *Entamoeba* Casagrandi and Barbagallo, 1895, is available as a generic name for *coli* and congeneric species at the same time that *Endamoeba* Leidy, 1879, is used for *blattae* and congeneric species.

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# THE STORAGE OF GLYCOGEN IN THE TEMNOCEPHALOIDEA

WILFRED FERNANDO
University of Ceylon, Colombo, Ceylon

## INTRODUCTION

The storage of glycogen in the body of the TEMNOCEPHALOIDEA has not so far been studied and the purpose of the present paper is to fill in this gap in our knowledge of carbohydrate metabolism in this group of PLATYHELMINTHES. The work was done entirely on adult animals, no attempt was made to study the distribution of glycogen during embryonic development.

I wish here to acknowledge my indebtedness to Prof. A. Kandiah and Dr. Eric Fonseka for the privilege of carrying out this work in the Department of Chemistry of the University of Ceylon and also to Prof. A. W. Mailvaganam for helpful suggestions. To Dr. Bruce Baptist of the Department of Agriculture, Peradeniya, I am indebted for the material which made possible the completion of the work. I must thank Mr. P. A. S. Perera of the Department of Physics for technical assistance.

## MATERIAL AND METHODS

The work was done on two representatives of the TEMNOCEPHALOIDEA; *Caridinicola indica,* which measures when preserved, about 0.6 mm. and *Monodiscus parvus* which measures about 0.3 mm. Specimens were obtained from fresh-water prawns at different seasons of the year from the following places in Ceylon—Anuradhapura, Kandy, Karawanella, Jaffna and Veyangoda.

As it was not considered safe to rely on any one method, several methods were employed to demonstrate the presence of glycogen in the body of these worms. In all more than one hundred specimens were sectioned and stained.

The following fixatives were used: Absolute alcohol–formol; Carnoy; Bouin–Allen (Pasteel's method); Chromo-osmic-acetic (Zieglwallner's method); and 10% formaldehyde saturated with dextrose (Neukirch's method).

The material was embedded by the paraffin method and by the celloidin-paraffin method. Sections were cut at 5μ to 7μ.

In staining, Best's carmine was found most satisfactory and results obtained from it were checked by the iodine method, Bauer's fuchsin—sulphurous acid method, and Fischer's tannin–gentian violet method. As controls, slides were digested with saliva for one hour at 37° C and stained.

## GLYCOGEN STORAGE IN *Caridinicola indica*

The glycogen in the outer epithelium of the body divides it into two layers—an outer or superficial layer which is free of glycogen and, next the basement membrane, an inner layer which has a great concentration of glycogen in the form of granules.

The basement membrane stains very deeply, indicating by its intensity a great concentration of the carbohydrate. It was not possible to identify in what form glycogen is stored here.

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The connective tissues which form the greater part of the parenchyma contain glycogen in the form of fine granules and rodlets. A small quantity of glycogen is stored in the parenchymatous cells in the anterior and middle regions of the body. The intercellular spaces contain a glycogen-rich fluid, the granules being less prominent. It is possible that in these intercellular spaces the carbohydrate is in the process of transference to the different parts of the body.

The muscles are a very important place for the storage of glycogen. Those investing the reproductive organs contain large quantities and stain very intensely. Of the muscles of the body wall, the diagonal muscles in the ventral region of the body and the circular muscles at the anterior region have little glycogen, but elsewhere glycogen is present in the form of granules in the plasma of the muscle cells. The longitudinal muscles in every part of the body contain large quantities of glyco-

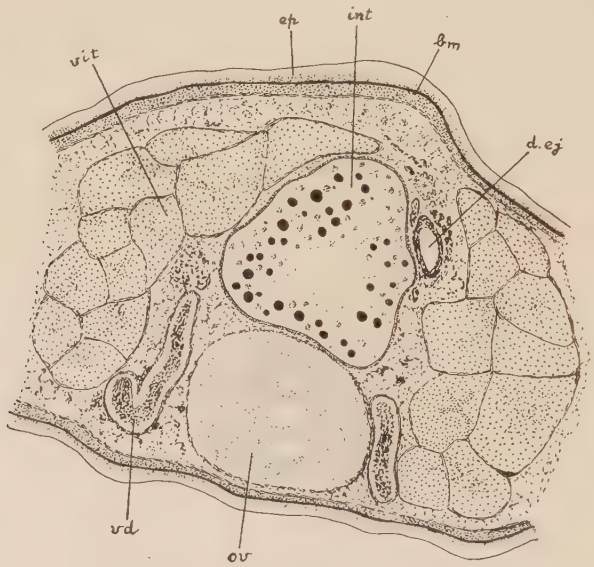


FIG. 1. Transverse section of *Caridinicola indica* passing through the region of the germarium. (Leitz ocular 16, obj. 6L) (Carnoy: Best's carmine). bm, basement membrane; d.ej, ductus ejaculatorius; ep, body epithelium; int, intestine; ov, germarium; vd, vas deferens; vit, vitelline gland.

gen, but the size of the muscle cells did not allow a very detailed study of the distribution.

The muscular posterior suckers have glycogen deposits, but the smaller, anterior suckers at the base of the tentacles are glycogen-free. On the other hand, the tentacles themselves contain glycogen granules. Numerous glands are present in the posterior suckers and in the tentacles and in these, glycogen is stored in the form of deeply staining granules or rodlets.

In the alimentary canal, the muscular pharynx (Fig. 3, ph) has glycogen deposited in the muscle fibres in the form of granules; at the posterior end of the pharynx these glycogen granules form a very characteristic, compact mass, contrasting that region sharply from the rest of the alimentary canal. The pharyngeal glands, which lie on the side of the pharynx, contain much glycogen. In the epithelial cells of the intestine, glycogen is present in the form of granules and also

as rounded masses inside vacuoles. The appearance of these glycogen masses in vacuoles in the intestinal epithelium is interesting (Fig. 1, int.). Some of them stain red, the others stain very lightly (pink), as compared with the glycogen in the muscles and it appears possible that with the absorption of food material, glycogen is synthesized in the epithelial cells and transported by way of the vacuoles to the outside and thence through the intercellular spaces of the parenchyma to the various regions of the body. The thin muscular coat investing the intestine, like other muscles in the body, has a deeply staining glycogen deposit.

Throughout the excretory system glycogen is present, distributed diffusely in the plasma of the excretory tubules. The staining is light in the tubules, but in the two excretory vesicles (Fig. 2) lying at the sides of the pharynx, the glycogen stains deeply and granular deposits can be seen. In these vesicles glycogen is deposited not only in the wall (as in the case of the excretory tubules) but also inside the lumen.

FIG. 2. Excretory vesicle of *Caridinicola indica* showing glycogen inside the vesicle and in the wall. (Leitz ocular 25, obj. 9A) (Carnoy: Best's carmine).

In the nervous system (Fig. 3) glycogen is found in the cerebral mass or brain in the form of fine, lightly staining granules and these granules can be traced to the nerves just as they issue out from the brain. More posteriorly the fine nerves are more or less obscured in the parenchymatous tissue so that it is not possible to state definitely whether glycogen is present throughout the nerves or not.

The muscular sheaths of the reproductive apparatus stain more intensely than do the muscles of the body wall, thereby indicating a more concentrated deposition of glycogen.

In the germarium (Fig. 1, ov), except for the very young oöcytes which are glycogen-free, the rest contain glycogen in the form of minute granules uniformly distributed in the cytoplasm. The epithelial investment of the germarium has no glycogen. The reservoir, into which the germarium opens, is a vesicle containing spermatozoa and yolk cells. Its epithelium has no glycogen, but the mass of spermatozoa found inside the lumen give a very dark glycogen stain. The function of the reservoir has been discussed in a previous paper (Fernando, 1934) and now it is apparent that the surplus spermatozoa are absorbed by the intestinal cells by way of the reservoir canal and the carbohydrate is passed into the body. The rest of the female apparatus consists of the oviduct, ootype and the atrium. In these, there is

general agreement in that the epithelium is always free of glycogen but the spermatozoa which travel along the female duct give the glycogen reaction. The unicellular glands which open into the oviduct throughout its entire length, contain glycogen granules which stain very deeply. Control slides, digested with saliva and stained by Best's carmine, do not take up the stain, so that it is evident that these glands contain glycogen.

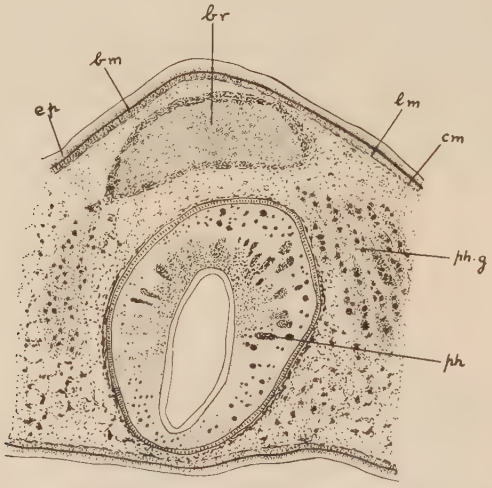


FIG. 3. Transverse section of *Caridinicola indica* passing through the brain and the pharynx. (Leitz ocular 16, obj. 6L) (Carnoy: Best's carmine). br, brain; cm, circular muscles; ep, body epithelium; lm, longitudinal muscles; ph, pharynx; ph.g, pharyngeal gland.

The vitelline glands contain glycogen (Fig. 1, vit). The membrane investing the follicles has rich deposits of glycogen granules; in the vitelline cells it is deposited as very small, uniformly distributed granules, which in some places become more concentrated and appear dark.

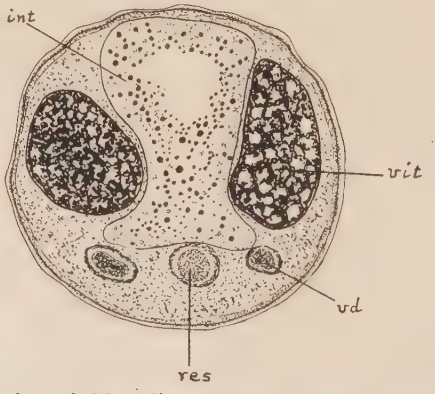


FIG. 4. Transverse section of *Monodiscus parvus* passing through the reservoir. (Leitz ocular 16, obj. 6L) (Carnoy: Best's carmine). int, intestine; res, reservoir; vd, vas deferens; vit, vitelline gland.

The envelope of the testes contains glycogen, but the developing sperm cells are glycogen-free. The mature spermatozoa, however, have a rich deposit of glycogen. The bundles of spermatozoa inside the testes give a very sharp contrast to the re-

maining unstained portions. The vasa deferentia and vesicula seminalis contain glycogen-staining spermatozoa. The epithelial parts of the vasa deferentia and the chitinous portion of the ductus ejaculatorius do not contain glycogen. The muscular sheaths throughout the male reproductive apparatus contain glycogen.

### GLYCOGEN STORAGE IN *Monodiscus parvus*

The distribution of glycogen in *Monodiscus parvus* resembles closely that of *Caridinicola,* but with few differences. The glands of the posterior sucker and of the tentacles give a more intense stain than in *Caridinicola.* There is more glycogen in the alimentary canal of *Monodiscus;* this glycogen is present as granules and in vacuoles uniformly distributed in the intestinal epithelium. The glycogen vacuoles here are comparatively larger and more numerous. The brain contains glycogen but the excretory system of *Monodiscus* seems to be glycogen-free. In the reproductive system, *Monodiscus* resembles very closely *Caridinicola* in regard to the distribution of glycogen, but the vitelline glands of the former has a richer deposit which stains very intesely (Fig. 4). In brief, although *Monodiscus parvus* is a smaller worm than *Caridinicola indica,* glycogen is stored in comparatively greater quantity than in the latter (judging by the intensity of the stain).

### DISCUSSION

The closest allies of the TEMNOCEPHALOIDEA are the TREMATODA and there is only one paper dealing with the distribution of glycogen in that group (Ortner-Schoenbach, 1913). The differences in regard to glycogen storage in the two groups may be mentioned. While the intestinal epithelium of trematodes is glycogen-free, that of temnocephalids contains glycogen, and in this the latter resemble the nematode *Ascaris* (Kemnitz, 1912). The trematodes are reported to lack glycogen in the nervous and excretory systems, but in the presence of glycogen in these two systems, the TEMNOCEPHALOIDEA resemble the ACANTHOCEPHALA as reported by von Brand (1939). The absence of glycogen in the excretory system of *Monodiscus* is noteworthy, but at present no explanation can be offered, nor can we explain why *Monodiscus* stores a relatively larger quantity of glycogen than does *Caridinicola,* which is a larger worm living together with the former on fresh-water prawns.

### SUMMARY

1. Glycogen is stored in almost all the organs of the TEMNOCEPHALOIDEA.

2. It is absent or found in negligible quantities in the outer layer of the body epithelium, in the diagonal muscles and in the epithelial lining of the reproductive ducts.

3. The nervous system stores glycogen.

4. Glycogen is found in the excretory system of *Caridinicola* but not in *Monodiscus.*

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# DESCRIPTIONS OF TWO NEW SPECIES, *PARATRICHOBIUS ANDUZEI* AND *NYCTERIBOSCA FRANCLEMONTI* (STREBLIDAE, DIPTERA, PUPIPARA)

ROBERT MATHESON

Jobling (1936) divided the family STREBLIDAE into four sub-families,—the NYCTERIBOSCINAE, the ASCODIPTERINAE, the TRICHOBIINAE, and the STREBLINAE. The genus *Paratrichobius* is placed in the TRICHOBIINAE. In 1939b Jobling described the known species of *Paratrichobius*—the type species *P. longicrus* (Ribeiro, 1907) and redescribed *Speiseria dunni* Curran as *P. dunni* (Curran). In the same publication he places the genus *Speiseria* Kessel (1925) as a synonym of *Synthesiostrebla* Townsend (1913). At the present time the genus *Paratrichobius* contains only the two above mentioned species. *P. longicrus* is reported from bats in Brazil, Paraguay and Mexico; *P. dunni* from bats in Panama. Some time ago I received three Streblids collected from bats (species not recorded) at San Estaban, Venezuela, by P. Anduze. These prove to be the third species for this genus.

*Paratrichobius anduzei,* new species

*Female:* Length 3.36 mm from tip of palps to tip of wings. Head strongly sclerotized with numerous stout, curving spines on the dorsal surface; eyes present, each consisting of eight facets; palps (Fig. 1, d) broadly rounded with one very long spine at outer apical point, several shorter ones and numerous short, setae-like spines; antennae present. Ventral surface of the head with a row of stout spines on each side of the rostrum membrane. Theca of labium cordiform, with four strong setae in front and a few scattered smaller ones (Fig. 1, e).

Prescutum (Fig. 1, a) somewhat pointed in front with a shallow indentation at the point of origin of the median suture; near the anterior margin is a row of short, stout spines; each antero-lateral corner with four or five stout, short spines; each lateral margin with a row of short, stout spines; area in front of transverse suture with small, scattered setae and a row of stouter setae in front of transverse suture. The transverse suture appears incomplete near the center and is not joined by the median suture. Scutum with numerous scattered small setae and a row of stout, spine-like setae just in front of the scutellum. Scutellum with sinuous posterior border and with four spines, the two posterior stout and very long; the anterior lateral in position and comparatively small. Legs stout; hind legs nearly three times as long as forelegs. Femora of prolegs each with a diagonal row of stout, spine-like setae and several smaller ones near them (Fig. 1, c); numerous short setae scattered over the femora; front tibiae with short setae and four long spines on outer margin.

Wing of female 2.6 mm in length; width at anterior cross-vein 0.8 mm.

Sternum narrowed in front and projects between and below the front coxae (Fig. 1, b) and is clothed with numerous, short, stout setae except the posterior margin which is provided with a row of stout spines. Ventral and lateral membranous parts of the abdomen with numerous small setae. The posterior border of tergite 1+2 interrupted in the middle and with a few fine setae; lateral borders enlarged and bear numerous, recurved, stout spines. Apical cone of abdomen with numerous long setae.

*Male:* The male closely resembles the female. The abdomen is more cylindrical and terminates in a long cone within which lie the genitalia.

*Holotype:* Female; paratypes, two males. Host, bats (species not recorded); collected at San Estaban, Venezuela, by P. Anduze on December 19, 1939. Types and paratypes in the collection of the Department of Entomology, Cornell University. I take pleasure in dedicating this species to the collector.

This species is easily distinguished from the two previously described species by the arrangement of the spines on the femora of the forelegs, by the shape of the mesonotum and the arrangement of its clothing setae, by the arrangement of the

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spines on the scutellum, by the shape of the sternum and the abdomen with its much larger and stouter spines on tergite 1 + 2.

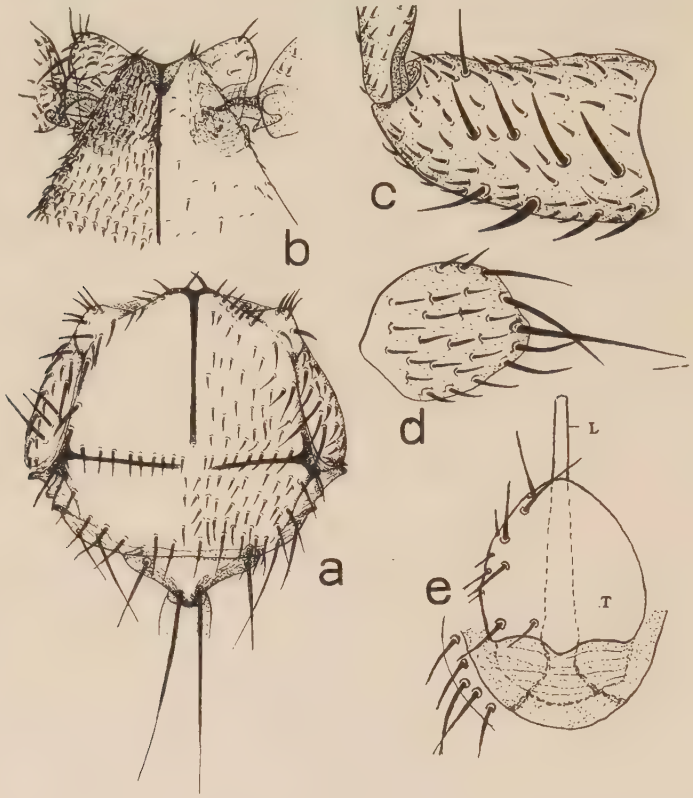


FIG. 1. *Paratrichobius anduzei.* a. Dorsal view of the thorax; the clothing setae are shown on only one side. b. Anterior part of the ventral surface of the thorax (Sternopleura of Jobling) projecting between and over the coxae. c. Femur of the foreleg showing the arrangement of the stout spines. d. Ventral view of the palpus. e. Ventral view of portion of head to show the theca and labella. L, labella; T, theca.

## THE GENUS *Nycteribosca* SPEISER

Jobling (1934) monographed this genus, describing seven new species and redescribing the other eight known species; he was unable to place *N. diversa* Frauenfeld. Since then the same author (1934, 1936a, 1936b) has described three new species—*N. minuta* from the Russell Islands, Solomon Islands; *N. bequaerti* from Tanganyika, Africa; and *N. scutellaris* from the Fiji Islands. Recently I received five specimens taken, presumably, from bats on Guadalcanal Island or the immediate vicinity by John Franclemont. This proves to be a very distinct species and is here described.

### *Nycteribosca franclemonti,* new species

*Female:* Length 3.17 mm (from tip of palps to tip of wing). Head (Fig. 2, d) not darker than the body; eye distinct, large but not projecting laterally; each laterovertex with spines, nearly all of the same size and curving; a group near apex stouter and longer; mediovertex broad; postvertex a perfect circle enclosing two to four spines (three in the type female; four in the male); occiput with small setae behind the postvertex; sides of the head with strong but shorter spines; ventral surface of the head with stout spines. Palps prominent, longer than broad,

each with three long spines and many shorter ones. Antenna prominent, the arista stout and bears four branches. Theca of the labium nearly as broad as long, with many small setae.

Thorax (Fig. 2, a) circular as viewed from above, as broad as long. The mesonotal suture fades out near the center. The mesonotum is densely clothed with long, spiny setae; those on the anterior half are about one-third to one-half as long as those on the posterior half. The scutellum is evenly rounded behind and bears numerous long setae; a row of much shorter setae along the posterior border. Ventral surface of thorax (Fig. 2, b) clothed with short setae. Legs are all strong and armed with stout setae on the dorsal surface; ventral surface with short, hair-like setae. Wing measures 2.24 mm in length; width at the anterior cross-vein 0.93 mm. Venation as shown in Fig. 2, c. Veins 3 and 4 diverge towards the apex of the wing.

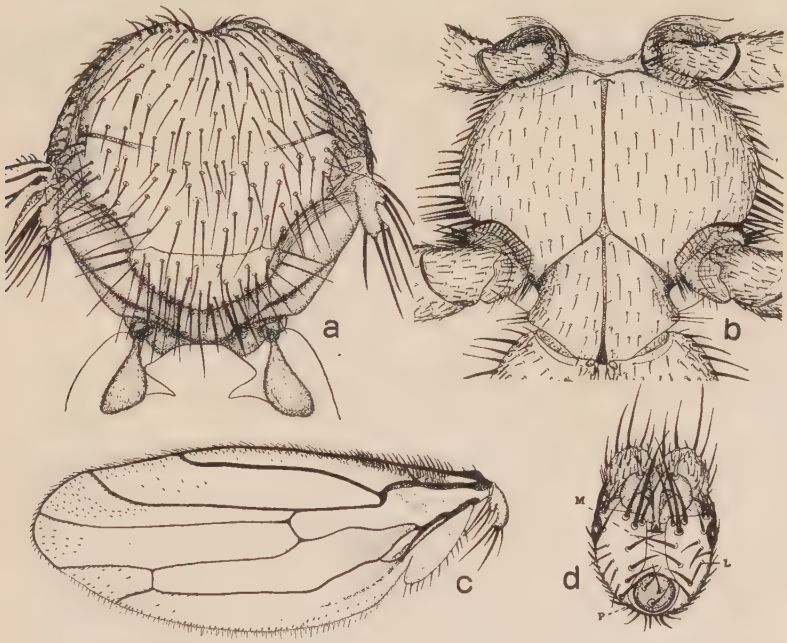


FIG. 2. *Nycteribosca franclemonti.* a. Dorsal view of the thorax. b. Ventral view of the thorax. c. The wing. d. Dorsal view of the head. L, laterovertex; M, mediovertex; P, postvertex.

Abdomen stout with dense masses of long hairs on each side of the bare part; each hair appears as if a series of fine parallel wires were twisted to a fine point. Tip of abdomen broadly conical; near the middle of cone on dorsal surface are four long setae; ventral surface with four long setae near middle and two before apex. Ventral surface of abdomen densely clothed with short stout setae.

The male is as large as the female and the only recognizable difference is the structure of the tip of the abdomen which bears the genitalia.

*Holotype:* Female; paratypes, 1 male and 3 females. Host a large bat. From Guadalcanal Island. J. Franclemont, collector. All specimens in the collection of the Department of Entomology, Cornell University.

This species runs to the group *pretiosa, amboinensis* and *surcoufi* in the key by Jobling (1934). It is readily separated from them by the circular postvertex, the characteristic setae of the scutum and scutellum and the density and length of the hairs of the abdomen. In the female the apical cone of the abdomen is different.

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# EXPERIMENTAL INFECTION OF SOUTHERN CALIFORNIA MOSQUITOES WITH *WUCHERERIA BANCROFTI*

OLIVER K. SCOTT, Lt., (MC), USNR, CHARLES S. RICHARDS, MA PhM1/c, AND ELWOOD A. SEAMAN, PhM2/c

Numbers of military personnel have returned from the South Pacific Island area showing early stages of infection with *Wuchereria bancrofti*. Practically none of these men has ever shown microfilariae in the blood stream. Since their exposure was short, it is very doubtful that any of them will ever have demonstrable microfilariae in their peripheral blood stream. However, it is of some interest to determine which, if any, of the mosquitoes of hitherto uninfected parts of the country could become carriers of this disease. In this investigation, all the common mosquitoes of the San Diego, California, area were tested for transmission. This was made possible by obtaining an 18-year-old Negro Navy mess attendant, born in Frederickstad, St. Croix, Virgin Islands. He had spent all his life in Frederickstad except for parts of 6 years which were spent in San Juan, Puerto Rico, until he entered the Navy in November, 1943. At that time it was noted there were microfilariae in a night blood smear. At the time of these examinations there were up to 20 microfilariae per night thick smear. He denied ever having had any symptoms referable to filariasis.

Adult mosquitoes of seven different species were caught in the neighborhood of San Diego, and two species, *Aedes taeniorhynchus* and *Culex stigmatosoma,* were hatched from pupae. All were exposed to the infected subject at approximately 2200 (i.e., 10 P.M.), but three species would not feed (see Table I).

TABLE I. *Results of dissections of mosquitoes fed on blood containing microfilaria*

| Mosquito | Dissected on death of mosquito before 17th day after feeding | | Dissected alive on 17th day or longer | |
|---|---|---|---|---|
| | Infected | Not infected | Infected | Not infected |
| *Culex erythrothorax* ..... | 14 | 11 | 18* | 12 |
| *Culex quinquefasciatus* ..... | 4 | 2 | 17† | 3 |
| *Culex tarsalis* ..... | 0 | 0 | 1‡ | 2 |
| *Aedes taeniorhynchus* ..... | 10 | 28 | 0 | 4 |
| *Anopheles maculipennis* ..... | 0 | 1 | 0 | 6 |
| *Anopheles pseudopunctipennis* | 0 | 1 | 0 | 0 |
| *Culiseta incidens* ..... | Would not feed on subject | | | |
| *Culiseta inornata* ..... | Would not feed on subject | | | |
| *Culex stigmatosoma* ..... | Would not feed on subject | | | |

Total dissections—134.
* Eight of these had larvae in the proboscis.
† Thirteen of these had larvae in the proboscis.
‡ This one had larvae in the proboscis.

It was found in those mosquitoes which became infected that the infective stage of the larva did not appear until the 15th to 16th day after the blood meal, thereafter mosquitoes were not sacrificed until the 17th day.

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(This work was done under the auspices of U. S. Navy Epidemiology Unit 80, Lt. Comdr. G. R. Underwood, Commanding.)

A list of species and number of mosquitoes and results after feeding are listed below. Summarized results are also shown in Table I; Figs. 1 and 2 show developmental stages of the larvae in these mosquitoes.

*Culex quinquefasciatus* Say (= *C. fatigans*):[1] 26 specimens fed on the subject were dissected. Six died before 17 days and of these 6, 4 had developmental stages of the larvae in the thorax. Of the 20 specimens that went through 17 days, 17 had the infective stage in the head or thorax and in 13 cases the mature larvae were seen in the proboscis itself. Three specimens were negative.

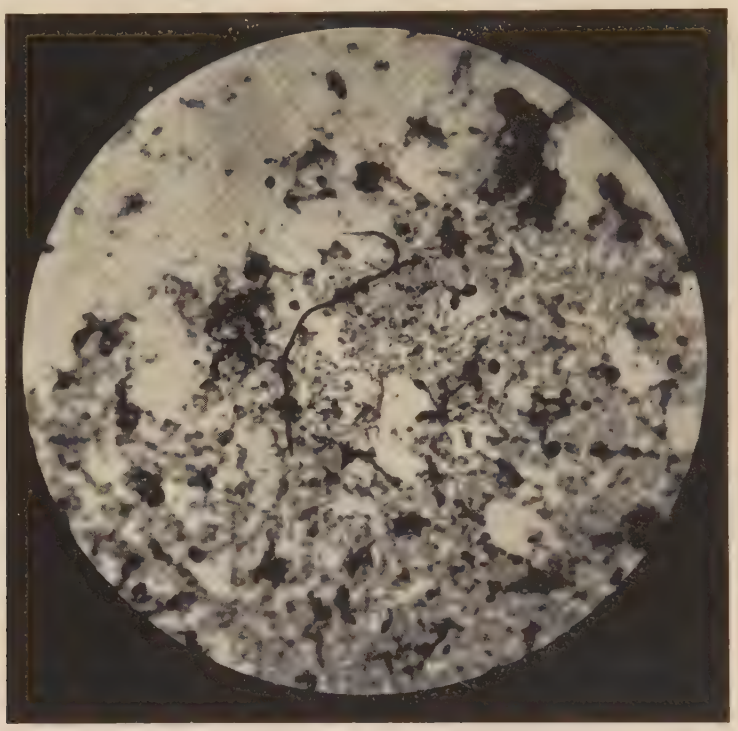

FIG. 1. Sheathless larvae in abdomen (outside stomach) of *Aedes taeniorhynchus*, 17 hours after feeding.

*Culex erythrothorax* Dyar: 56 specimens fed on the subject were dissected. Twenty-six died before 17 days. Of these 26, 14 had developmental stages of the larvae in the thorax. Of the 30 specimens that went through 17 days, 18 had the infective stage in the head or thorax and in 8 cases the mature larvae were seen in the proboscis itself. Twelve specimens were negative.

*Culex tarsalis* Coquillett: Due to the season of the year, late summer, only three specimens were obtained and fed. All of these survived 17 days and one had infective stages of the larvae in the thorax, head and proboscis. The other two specimens were negative.

*Aedes taeniorhynchus* (Wiedemann): 42 specimens fed on the subject were dissected. Thirty-eight died before 17 days and of these 38, only 10 had develop-

[1] Identification of this species made by the courtesy of Dr. Alan Stone of the U. S. National Museum, Washington, D. C., and Dr. W. C. Reeves, of the George Williams Hooper Foundation, San Francisco.

mental stages of the larvae in the thorax. The four that survived 17 days were all negative.

*Anopheles maculipennis freeborni* Aitken: Seven specimens fed were dissected. All specimens were uninfected and all but one survived for 17 days.

*Anopheles pseudopunctipennis* Theobold: This species would not feed except one specimen which did not become infected.

*Culex stigmatosoma* Dyar: Although very large numbers, several hundred, were exposed to the patient, none fed.

*Culiseta inornata* (Williston) and *Culiseta incidens* (Thomson), the other two common mosquitoes in this area, do not ordinarily feed on humans, and the specimens obtained did not feed on this individual.

FIG. 2. Larva in thorax of *C. quinquefasciatus*, 7 days after feeding.

## CONCLUSIONS

If persons with circulating microfilariae were present in Southern California, the local mosquitoes *Culex quinquefasciatus, Culex erythrothorax,* and probably *Culex tarsalis* would be vectors of the Bancroftian filariasis. Because so many mosquitoes died under laboratory conditions the facts are inconclusive as to *Aedes taeniorhynchus,* but what evidence there is indicates that it would be a less efficient vector than the above-mentioned three species. *Anopheles maculipennis freeborni, Anopheles pseudopunctipennis* and *Culex stigmatosoma,* and the Culisetas, would be unlikely vectors.

# *PAGURITHERIUM ALATUM* N. G., N. SP., AN ENTONISCIAN PARASITE OF *PAGURUS LONGICARPUS*

EDWARD G. REINHARD
Department of Biology, Catholic University of America

The ENTONISCIDAE constitute a very specialized family of isopod crustaceans (suborder EPICARIDEA), whose members are found only within the haemocoel of brachyuran and anomouran crabs, where they are invested by an integumental sheath that communicates with the exterior of the host. Until recently no representatives of this family had been reported from North America. In 1938 Pearse and Walker (1939) found two species in xanthid crabs from North Carolina and Prince Edward Island which proved to be new species of the genus *Cancrion.* In 1943 the writer discovered another entoniscid at Woods Hole, Mass. This parasite infects hermit crabs and represents a new species and new genus. Moreover, it is peculiar in communicating with the exterior not as others are reported to do, by way of the host's branchial chamber, but through an opening in the head or eyestalk of the crab.

The host of this entoniscid is *Pagurus longicarpus* Say, a littoral species extremely abundant in the Woods Hole region. All the crabs were collected from the beach fronting the Yacht Club in Great Harbor. Of some 4600 taken from this locality during August and September, 1943, 38 crabs were found to contain one or more adult female parasites, an infection rate of 0.8 per cent.

A hermit crab containing an adult entoniscid can be recognized with practice by the pale appearance of the abdomen and the scanty liver tissue. Often the eggs and embryos of the parasite are discernible through the semi-transparent integument of the host's abdomen. With the exception of a hundred or so apparently normal specimens the only crabs dissected were those showing external indications of the presence of parasites and therefore young stages were not encountered. A complete examination of all crabs to uncover those having the "asticot" stage would undoubtedly have raised the percentage of parasitism but the task would have been very arduous since the infection rate with all stages could not have exceeded 1.5 or 2 per cent.

The adult male and female parasites resemble most closely the males and females of the genus *Entoniscus.* Similarity to *Entoniscoides* is less marked. In important respects, however, this entoniscid differs from these and other known genera of the family and a new genus is required to contain it. This genus we propose to call *Pagurítherium* (*Pagurus* + *therion,* a beast). The specific name *alatum* is in reference to the first pair of oöstegites of the female which resemble great wings.

Cotypes of this new entoniscid have been deposited in the U. S. National Museum.

## RELATION OF THE PARASITE TO THE HOST

The full-grown parasite is about two-thirds as long as the crab. The body proper with its enormously developed brood chamber occupies most of the available space in the abdomen of the host and its thin elongated pleon reaches forward

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through the host's thorax into the most anterior portion of the head (Fig. 1.) Not only is its position reversed with respect to the host but also inverted since the parasite's dorsal surface is directed towards the ventral surface of the host.

In traversing the thorax the pleon passes beneath the heart, stomach and brain but since it is merely a slender tube the organs of the crab in this part of its body are not displaced or harmed. In the abdomen, however, where the bulk of the parasite is located, the hepatopancreas of the crab and its reproductive organs are greatly reduced. In some cases only by means of serial sections can any trace of the gonads be found.

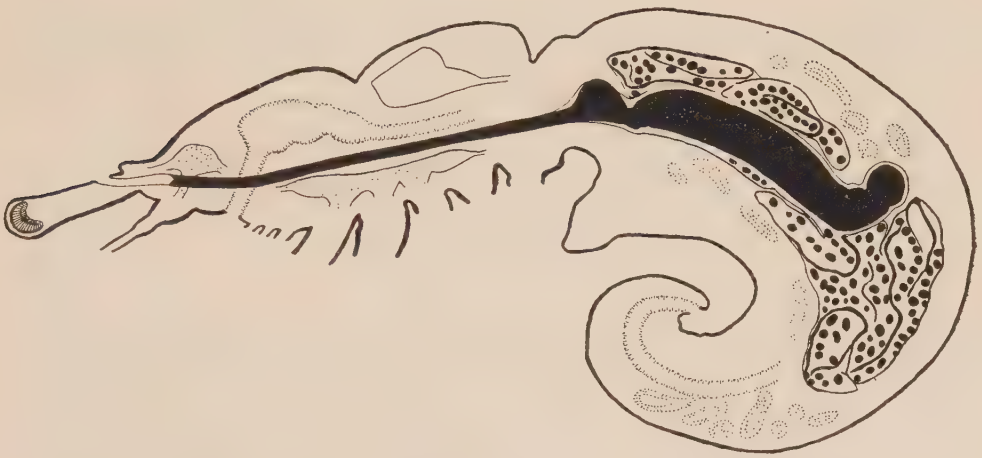


FIG. 1. Diagram of a hermit crab showing the parasite, *Paguritherium alatum, in situ.* Note its communication with the exterior through the wall of the crab's eyestalk. The parasite proper is represented in solid black. Surrounding it is the enclosing sheath and, to the right, the brood pouch of the parasite filled with embryos.

Throughout its entire extent the entoniscid is invested in a thin sac closely molded to the contour of its body. This sheath is derived from the hypodermis of the host and is seen distinctly as a separate entity only near its point of origin at a perforation in the body wall of the crab. There it forms a short hollow stalk since the tip of the parasite's pleon does not quite extend all the way to the aperture. The integumental sheath of entoniscids arises, as has been shown by Giard and Bonnier (1887) and others, at the time when the larval entoniscid punctures the integument of the crab to gain entrance into the host's body and spreads inwards progressively as the parasite penetrates further and grows larger. The sac thus formed shuts off the animal from direct contact with the crab's viscera but at all times retains its communication with the exterior at the original point of invagination.

In the case of all previously studied entoniscids this aperture occurs in the inner wall of the branchial chamber of the host. In the present animal, however, it lies in the head or eyestalk of the crab. The precise location was determined in ten infected crabs by careful dissection or by means of serial sections and gave results as follows:

| | |
|---|---|
| In the corneal surface of the eyestalk | 2 |
| In the distalf half of the eyestalk | 3 |
| In the proximal half of the eyestalk | 1 |
| At the base of the eyestalk | 2 |
| At the base of the second antenna | 2 |

The aperture in the case of the present species is a somewhat oval opening measuring only 0.15 mm in greater diameter and about 0.10 mm in lesser diameter. The inturning of the hypodermis at the margins of this opening is accompanied by an inturning of part of the chitin layer which thus forms a ring over the inner surface of the sheath for a slight distance back from the opening. To this thickened portion Giard and Bonnier gave the name "casque" or "calyce chitineux." In the present entoniscid it is not as well developed as in the genera described by them. Its extent is only about 0.2 mm and its thickness about 10 μ.

*Paguritherium* new genus

*Female.*—Body slender and lacking ovarian processes; brood chamber open; four pairs of well-developed oöstegites and a rudimentary fifth pair; margins of oöstegites not deeply notched; oöstegite I the largest, consisting of a large ascending lamella folded laterally into an inner and outer plate, with descending lamella very short. Pleon long, practically uniform in diameter throughout, with five pairs of saber-like appendages; telson invaginated.

*Male.*—Antennules tuberculiform; pereiopods stump-like and spinose anteriorly; telson unforked, linguiform and spinose posteriorly.

*Genotype.*—The species described below.

*Paguritherium alatum* new species

The Female (Figs. 2–4)

The cephalogaster is a swollen bilobed structure as in other ENTONISCIDAE, with antennules and antennae in the form of thickened pads fused lip-like around the mouth. The only prominent mouthparts are a pair of fleshy maxillipeds. Maxillae are lacking and the mandibles are extremely minute. A possible peculiarity of the cephalogaster of this species is the comparative narrowness of the hemispheres.

The thorax or perion, which is almost entirely filled by a highly developed ovary and the hepatopancreas, is a smooth narrow cylinder without ovarian processes. It bears the oöstegites which form the framework for a brood chamber of the open type. Over this framework is stretched the integumental sheath so that a capacious pouch is formed to hold the developing eggs and embryos.

Only the first four pairs of oöstegites are concerned in brood pouch formation. The two lamellae of each of these pairs overlap in the mid-ventral line and the posterior margins of oöstegites II and III also overlap the anterior margins of oöstegites III and IV, respectively. The lamellae are leaf-like in appearance, exhibiting a venation which usually takes the form of three main veins with subdividing branches. At their free margins they are crispate and have a few shallow indentations.

Oöstegite I consists of an ascending lamella of considerable length and a very short descending lamella. The ascending branch rises over the cephalic region like a great wing and is folded laterally upon itself so that a shorter outer leaf and a very much longer inner leaf are differentiated. A descending branch and possibly a transverse branch are present but both are quite rudimentary. This oöstegite is inserted near the upper end of the thorax and between it and the insertion of the next oöstegite there is a conspicuous gap.

Oöstegite II consists of a single ascending lamella which extends upwards from about the middle of the thorax without quite reaching the cephalogaster. It is about two-thirds as long as the ascending branch of oöstegite I and somewhat narrower.

The third oöstegite is about half as long as its predecessor and the fourth is still shorter, but both diminish only slightly in breadth. They project ventrally also and are so close together that the latter is practically a lobe of the former. Oöstegite V is a narrow inconspicuous plate crowded out of position and folded against the dorsal surface of the thorax in front of the heart, thereby forming a pocket for the storage of sperm.

The pleon is sharply set off from the thorax and consists of five pleomeres and a telson. In the living animal it can be extended to a length exceeding that of the thorax but in preserved specimens it is always found strongly contracted, particularly in the region of somites III to V. At its junction with the thorax it is greatly dilated by the presence of the heart. Elsewhere it is a rather slender stalk-like structure of almost uniform diameter throughout, tapering only slightly at the posterior end. Each pleomere is provided with a pair of appendages in the form of pointed saber-like processes. The pleural lamellae, conspicuous structures in most ENTONISCIDAE, are here represented merely by a narrow membranous frill on the two proximal

segments. These segments are considerably longer than the others. The truncate telson is excavated at its distal extremity and crowned by a fringe of short marginal hairs.

The general color of the female is pale yellow, but the presence of mature embryos may give it a deep yellow or rusty appearance. The hepatopancreas has a red coloration and the oöstegites are whitish.

One of the larger specimens measured with brood pouch filled and enclosing sheath present had a total length of 15 mm including the pleon which measured about 5 mm. The greatest width at any one point was about 4 mm.

The female of this genus differs from *Etoniscus* Müller chiefly in having the anterior oöstegites greatly enlarged, with the second to the fourth decreasing in size, and the fifth pair rudimentary, while in *Entoniscus porcellanae,* the type species of the genus, all oöstegites are well developed, with the posterior pairs larger than the anterior ones.

### The Male (Figs. 5, 6)

The male is found inside the brood chamber of the female, and, as a rule, one male accompanies each female.

The male attains a length of about 2 mm. The specimen drawn measured 1.8 mm. The body color is pale with a few dark brown chromatophores. In general shape of the body and particularly in the stump-like appendages it resembles the male of *Entoniscus porcellanae,* a Brazilian species described by Müller (1862). It differs notably, however, in the shape, size and position of the antennules, which, in *E. porcellanae,* form prominent, antero-lateral, quadrangular expansions.

The head of the male is short and blunt and has antennules in the form of knob-like protuberances that project forward without reaching the frontal margin. Each is surmounted by about 10 short curved setae. The antennae are represented only by an inconspicuous tuft of bristles in front of the eyes. The mandibles, which are moderately narrow and notched at the tip, are enclosed in a suctorial cone as is usual in the ENTONISCIDAE. The opening of this cone is somewhat ellipsoidal with an acute mid-lateral expansion on each side. The surface of the lower lip is smooth, not covered with scale-like markings as occur for example in *Pinnotherion* or *Entionella.* Maxillulae could not be detected. The maxillae appear to be small wing-shaped lamellae meeting in the mid-line. These are concealed below broader leaf-like plates which represent the maxillipedes.

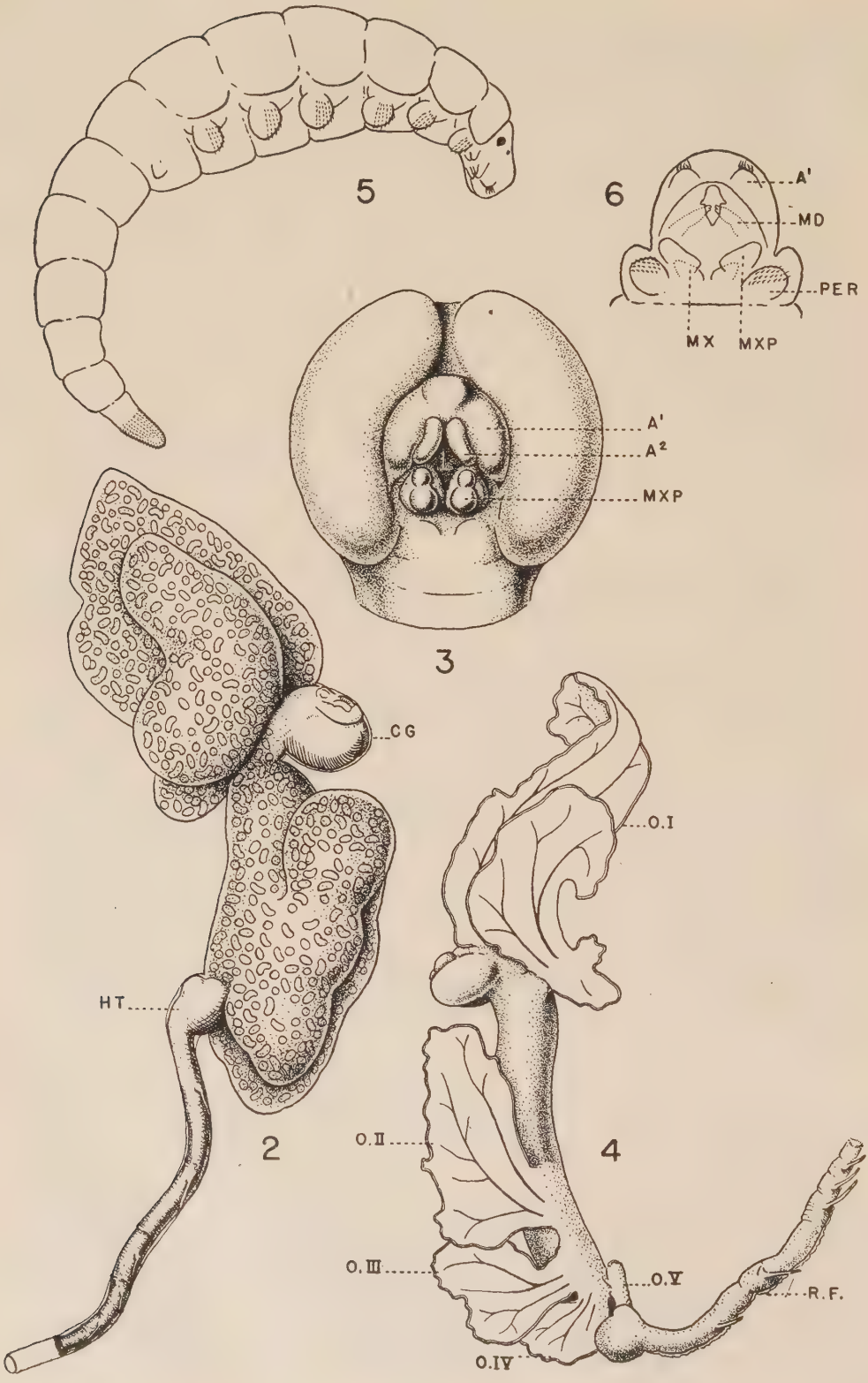
The rather plump thorax is composed of seven somites carrying six pairs of stump-like appendages on I to VI. These thoracopods (pereiopods) are all alike except that they increase slightly in size to the fifth pair, the sixth and fourth being about equal. Each consists of a broad, fleshy basal segment and a rounded protopodite which is a single undivided knob. The protopodite is provided on its anterior half with rows of short spines. The seventh thoracic segment bears a pair of smooth lateral tubercles. Miyashita (1940) regards similar protuberances on the seventh somite of the male *Entoniscoides* as rudimentary pleopods but they lack the basal piece and in the present instance the spines of the other pereiopods. It is evident that these are genital tubercles, homologous with the penis of *Priapion.*

The pleon consists of five segments and a telson, the latter tongue-shaped and undivided. The pleomeres are without appendages and diminish in size posteriorly. Spines are absent from the ventral side of the pleomeres but occur on the posterior half or posterior two-thirds of the telson.

### DEVELOPMENT

In previously described ENTONISCIDAE, with the exception of *Entoniscus* and *Entoniscoides,* the embryos in the brood chamber are all of the same age. Embryos of diverse stages are present in one brood of *Entoniscus porcellanae* according to Müller (1862), and hatching occurs in the epicarid stage. A Japanese species of *Entoniscus* is reported by Miyashita (1940, p. 153 footnote) to have a brood consisting of three different sorts of embryos. In *Entoniscoides okadai* two kinds of embryos make up the content of the brood chamber and development is abbreviated so that the larva hatches in the cryptoniscan stage (Miyashita, 1940).

The present species is like *Entoniscus porcellanae* in that a mixture of eggs in early development and advanced larval forms are invariably found in the same


5
6
A'
MD
PER
MX
MXP
A'
A²
MXP
3
CG
HT
2
O.I
O.II
4
O.III
O.V
R.F.
O.IV

brood pouch. It appears that a second and often a third batch of eggs is released, after a considerable time interval, into the pouch which already contains the developing embryos of a previous laying, so that two or three widely different stages are encountered together. The free-swimming larval stage which escapes from the brood chamber is epicarid in form. It does not differ materially in structure from that of *Pinnotherion vermiforme* described and figured by Atkins (1933). When ready for emergence it makes its way into the space between the integumental sheath and the pleon of the parent and crawls out through the aperture in the body wall of the crab with which the sheath is in constant communication.

The round unsegmented ova vary in diameter from 95 μ to 115 μ. Segmentation results in an oval embryo about 132 μ by 95 μ. Young epicarid embryos measure from 160 μ to 180 μ in length and 64 μ in dorso-ventral direction; older epicarids measure about 227 μ in length and 90 μ in breadth.

The last larval or cryptoniscan stage was not seen. All the cryptoniscan larvae found clinging to the surface of the host proved to be those of the bopyrid *Stegophryxus hyptius* with which *Pagurus longicarpus* is likewise parasitized at Woods Hole (Thompson, 1902; Reinhard, 1943).

## SUMMARY

*Pagunitherium alatum,* representing a new genus and species of parasitic isopods of the family ENTONISCIDAE, is described.

The genus is most closely related to *Entoniscus,* but differs from it, in the case of the female, by the greater enlargement of the anterior oöstegites and the reduction of the posterior ones, while, in the male, the antennules do not form antero-lateral expansions.

Unlike other known entoniscids, this species communicates with the exterior through an opening in the head or eyestalk of the host.

The host is *Pagurus longicarpus* Say. Thirty-eight hermit crabs were found at Woods Hole, Mass., parasitized with adult stages, an infection rate of 0.8 per cent.

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EXPLANATION OF FIGURES

Abbreviations

| | |
|---|---|
| $A^1$—antennules | O.I.—first oöstegite |
| $A^2$—antennae | O.II—second öostegite |
| CG—cephalogaster | O.III—third oöstegite |
| HT—heart | O.IV—fourth oöstegite |
| MD—mandibles | O.V—fifth oöstegite |
| MX—maxillae | PER—pereiopods |
| MXP—maxillipeds | R.F.—respiratory folds |

FIG 2. Adult female of *Pagunitherium alatum* with embryos present in brood pouch and enclosing sheath intact.

FIG. 3. The cephalogaster of a female.

FIG. 4. Adult female stripped of enclosing sheath and contents of brood pouch. The oöstegites are drawn only on the left side.

FIG. 5. Lateral view of a male of *Pagunitherium alatum.*

FIG. 6. Ventral view of the head region of a male.

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# METAMORPHOSIS OF THE FROG HOST AS A FACTOR IN CERCARIAL PENETRATION BY *GLYPTHELMINS QUIETA**

By W. Henry Leigh[1] and Harley J. Van Cleave[2]

*Glypthelmins quieta* (Stafford, 1900) is a digenetic trematode of the family Plagiorchidae whose marita occurs in the intestine of frogs (Leigh, 1937, et al). Several species of snails of the genus *Physa* serve as the first intermediate hosts for the alternate generations of *Glypthelmins*. Cercariae liberated from the body of a snail become encysted as metacercariae in the skin of frogs (Leigh, 1937, and in press; Rankin, 1944). The worms gain access to the intestine of the definitive host only when the cast skin bearing the encysted metacercariae is devoured by the frog. Thus the frog serves both as second intermediate host and definitive host for *Glypthelmins quieta*. This unusual situation is made possible by the fact that a frog devours its own cast skin. Active cercariae ingested by tadpoles and frogs are apparently destroyed in the digestive tract for they have never been found encysted in the viscera.

Preliminary observations and experiments had demonstrated the fact that the cercariae of *G. quieta* refuse to penetrate and become encysted in the skin of tadpoles of the same species of frogs which serve as normal intermediate hosts following metamorphosis. Habits of the cercariae, which usually swim upward toward the surface-film, would tend to avoid natural contact with tadpoles but would facilitate contact with frogs swimming or resting at the surface.

In repeated attempts, tadpoles of *Rana pipiens* and *R. catesbeiana* were exposed to thousands of cercariae of *Glypthelmins* in glass dishes. The reaction was invariably different from that observed with frogs. Upon contacting the skin of a tadpole, the cercariae became attached and crawled about rapidly, never stopping to initiate the movements characteristically performed in attempting penetration. Their uninterrupted movements gave the impression that they were "seeking" suitable spots which they never found. During this period of rapid crawling the cercariae were attached by the suckers only and in numerous instances vigorous movements of the tadpole were observed to dislodge them.

The difference in response of the cercariae to skin of tadpole and metamorphosed frog suggested the problem of determining experimentally the conditions under which penetration became possible and an analysis of these conditions. A series of tadpoles of *Rana catesbeiana* was used in these experiments.

In two instances tadpoles which had begun metamorphosis and had attained the hind legs were exposed to large numbers of cercariae of *G. quieta*. Although the tail had begun to degenerate, it was still present. Cercariae became encysted in the skin of the legs and body but none penetrated the skin of the tail in either experiment. These experiments suggested that metamorphosis, as it affects cercarial penetration, does not affect the entire body simultaneously but is accompanied

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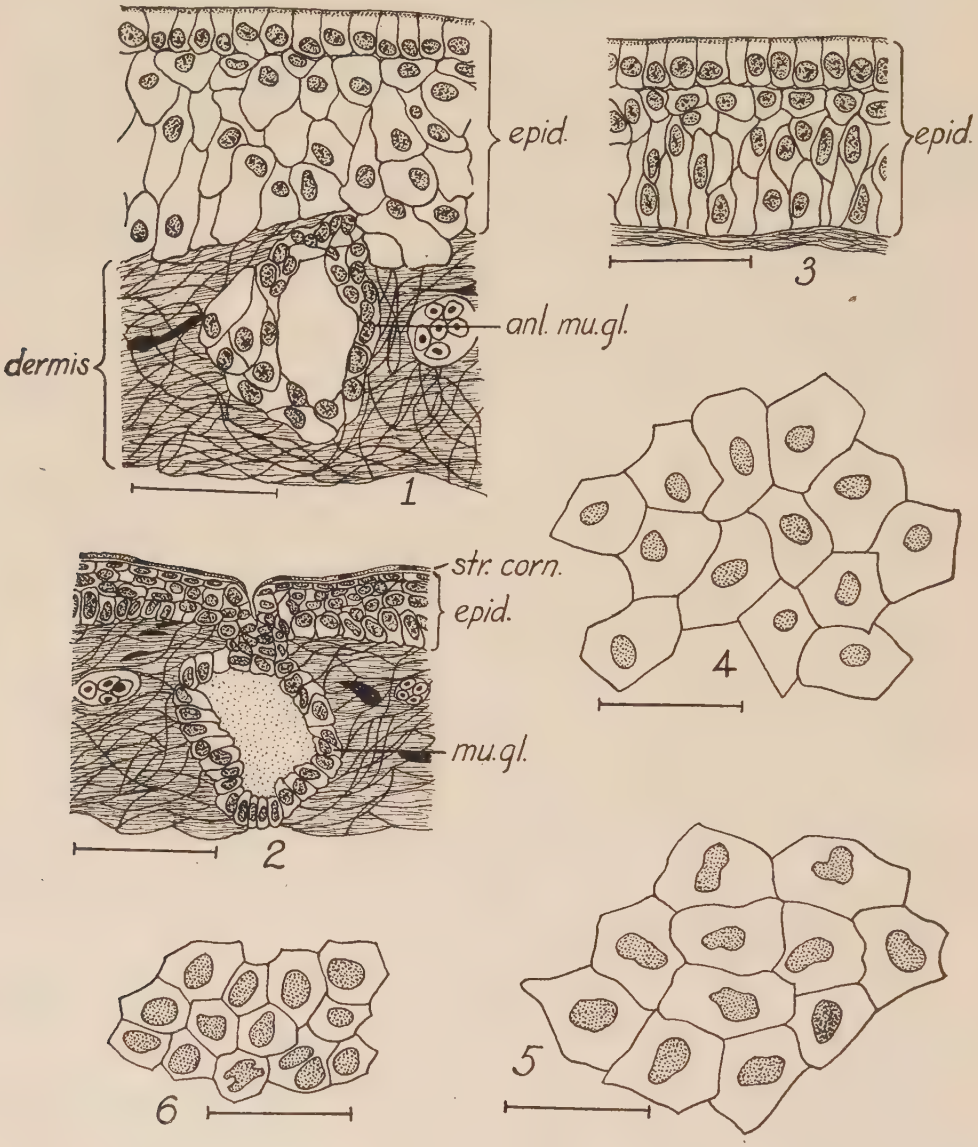
[1] Aviation Physiologist, United States Army Air Forces.

[2] University of Illinois, Urbana, Illinois.

## Explanation of Plate

All figures were prepared as preliminary camera lucida sketches which were redrawn by Katharine Hill Paul, scientific artist in the Department of Zoology of the University of Illinois. Each line indicating magnification has the value of 0.05 mm.




Integument of *Rana*

Fig. 1. Section through the body integument of a tadpole of *R. catesbeiana* before exposure to thyroid extract showing normal, unmetamorphosed larval skin. Note that the anlage of the mucus gland (anl. mu. gl.) has not acquired a duct. The external layer of the epidermis (epid.) has not yet become a stratum corneum.

Fig. 2. Section of the body integument of a tadpole of *R. catesbeiana* following exposure to thyroid extract. Note that the skin has undergone metamorphosis. The outer layer of cells (str. corn.) now forms a stratum corneum, the entire epidermis (epid.) has undergone transformation, and the mucus gland (mu. gl.) has attained connection with the surface.

Fig. 3. Section of tail of normal tadpole of *R. catesbeiana* showing unmetamorphosed epidermis (epid.).

by a series of progressive, coordinated morphological and physiological changes in the skin.

There is an extremely voluminous literature on normal and experimentally induced metamorphosis in AMPHIBIA, but most of this is concerned primarily with the conspicuous organic changes that mark the attainment of legs and loss of the tail. The histological changes accompanying metamorphosis of the tadpole are less obvious but are fully as significant. Microscopic examination of the skin reveals marked differences between that of the tadpole and the frog. The epidermis of the tadpole (Fig. 1) is much thicker than that of the frog (Fig. 2), chiefly because the component cells are larger. In the tadpole, cells of the outer layer are cuboidal to slightly columnar in form but upon metamorphosis the integument of the frog attains a covering layer of very flat, squamous epithelium designated as the stratum corneum. The large acinous glands, characteristic of the adult skin, are entirely lacking in the tadpole. In fact Wilder (1925) and Helff (1931) state that no anlagen of the acinous glands appear until just prior to the onset of metamorphosis. In the tadpole (Fig. 1) no ducts are formed to connect these anlagen with the external surface. One type of the acinous glands produces the mucus so distinctive of the skin of an adult frog. Although there are other minor differences between the skin of the tadpole and that of the frog the three here enumerated seem particularly significant: (1) change in the shape of the cells covering the body surface (Figs. 4, 5, 6), (2) attainment of gland openings, (3) the functioning of the mucus and other acinous glands. The differences here enumerated suggest that either structural or physiological changes in the skin might furnish the basis for explaining the difference in susceptibility to cercarial penetration. It seems probable that structural and physiological changes are both important.

That cercarial penetration of tadpole skin is not physically impossible is shown by the fact that in other species of XIPHIDIOCERCARIAE, fairly closely related to *Glypthelmins,* penetration of tadpole skin is accomplished regularly in carrying on the normal life cycle. Thus McCoy (1928), Talbot (1933), Byrd (1935) and McMullen (1935) have demonstrated life histories in which XIPHIDIOCERCARIAE pass through the skin of tadpoles and become encysted in deeper tissues. These observations would suggest that the stimulus or chain of stimuli leading to penetration may vary in closely related species of trematodes and that stimulation which will initiate penetration for one species may be ineffectual for another.

One set of observations seems to localize definitely the source of the stimulation to penetration within the skin of the metamorphosed frog. With only the cast skin from a metamorphosed frog in the container, cercariae have been observed to accomplish complete penetration from either side of the stratum corneum. Penetration of the cast-off skin was slower than normal penetration of the skin attached to the frog body. This might readily be explained by the fact that the detached skin allowed for lost motion more than the skin tightly drawn over the frog's body. The cercarial tail was dropped after penetration of the loose skin began and when the

FIG. 4. Surface view of a piece of cast skin from the body of an adult *R. pipiens,* showing relative size of cells and nuclei.

FIG. 5. Surface view of a piece of cast skin from the body of a tadpole of *R. catesbeiana* following exposure to thyroid extract.

FIG. 6. Surface view of a piece of cast skin from the body of a normally metamorphosed young individual of *R. catesbeiana.*

tailless cercaria fell through on the opposite side of the skin the stimulus for further attempt at penetration seemed lacking. There was no evidence of cyst formation following successful penetration of shed skin.

Since the functioning of the mucus glands is one of the most distinctive changes marking metamorphosis of the frog skin, a series of experiments was devised to see whether the mucus could be responsible for the stimulation of cercariae to penetration. There are no direct observations that would tend to indicate that penetration is by way of the openings of the acinous glands. In fact detailed observations by McMullen (1937: 236) upon the mechanics of penetration by another species of XIPHIDIOCERCARIAE stress the importance of the stylet as an excavating tool in piercing the body covering of a second intermediate host. Mucus scraped from the skin of a frog was spread on the body of a tadpole which was then subjected to cercariae of *Glypthelmins*. The carcariae gave no evidence of attempt at penetration. It is probable that most of the mucus was washed from the skin of the tadpole when it was returned to the water.

If mucus is the agent responsible for initiating the penetration responses of *Glypthelmins* cercariae there must be some specific substance other than the mucin present because the cercariae fail to penetrate the skin of salamanders, rich in mucus glands. Two series of experiments were carried on to test the potentiality of mucin as a stimulus to the cercariae. Mucin was extracted from the skin of *Rana pipiens* in 10% NaCl and was then precipitated in dilute acetic acid, washed and filtered. The dried extract was removed in thin sheets from the filter paper. These thin sheets, insoluble in water and about the thickness of the stratum corneum, were placed in water containing cercariae of *Glypthelmins*. Many of the latter attached themselves temporarily but made none of the characteristic movements associated with the act of penetration. It is possible that some specific substance capable of influencing action of the cercariae was destroyed during the preparation of the mucin. Mucin from another source was likewise tested in a series of non-conclusive trials. NaOH was used to extract mucin from ground parotid glands of sheep. When this was precipitated by weak acetic acid and placed on the skin of tadpoles it produced no observable change in the indifferent response of the cercariae.

A tadpole that was undergoing normal metamorphosis in the laboratory was exposed to cercariae after both pairs of legs had appeared although much of the tail still remained. Cercariae penetrated the skin of the legs and body and became encysted although they did not attempt to enter the skin of the tail. The encysted metacercariae were evident in the body and leg skin that was shed by this individual, and on autopsy it was found that some cercariae had penetrated into the deeper layers of the skin and were not sloughed off with the molt. Microscopic examination of pieces of the shed skin showed the flattened external layer of cells and many openings of acinous glands distinctive of the skin of the adult frog.

In another instance of a normally metamorphosing tadpole the skin of the hind legs contained a number of encysted metacercariae although none could be found in the skin of either the body or the tail. These results suggest that the metamorphosis of the skin is progressive and that a new adult organ such as the leg has a skin characteristic of the adult before the skin of the larval body transforms.

In order to observe the effects of accelerated metamorphosis of tadpoles upon the reactions of cercariae, young tadpoles of *Rana catesbeiana* were treated with

thyroid extract. In from 7 to 10 days the treated tadpoles showed a marked change in skin texture (compare Figs. 1 and 2) and in susceptibility to cercarial penetration. The outer layer of columnar cells on the body proper was replaced by a squamous epithelial layer of cornified cells identical in appearance with the stratum corneum of the mature frog. Cells of the deeper layer of the skin became much reduced in size, and the nuclei occupied a larger volume of the cells. These histological changes took place in the thyroid-treated frogs before the front legs and other gross evidences of metamorphosis had made their appearance. The hind legs had just begun to develop and the intestine retained its much coiled condition when the treated tadpoles began to molt a layer of skin that had every appearance of the skin of an adult frog. Following this experimentally induced metamorphosis of the skin, the cercariae of *Glypthelmins* were able to penetrate and become encysted as in the skin of normally metamorphosed frogs.

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# A NEW TREMATODE, *NEORENIFER CROTALI*, FROM THE RATTLESNAKE[1]

J. Teague Self

The specimens on which this paper is based were collected in March, 1942, from a specimen of *Crotalus atrox* Baird and Girard 1853, by Frank B. McMurry, wildlife technician, in the Wichita Mountains Wildlife Refuge.[2] Ninety specimens were taken from the lower lung, mouth and esophagus of the host. The latter was killed by drowning and since most of the flukes were found imbedded in the tissue of the lower lung it is assumed that those found in the mouth and esophagus were washed there, although this must remain a point in question. They were fixed in hot 70% alcohol and the ten *toto* mounts used in this study were stained in either Semichon's aceto-carmine or Mayer's paracarmine. One specimen was sectioned serially for study of the excretory system.

The specimens belong to the genus *Neorenifer* Byrd and Denton, 1938, and apparently to a new species. Appreciation is due Mr. McMurry for furnishing

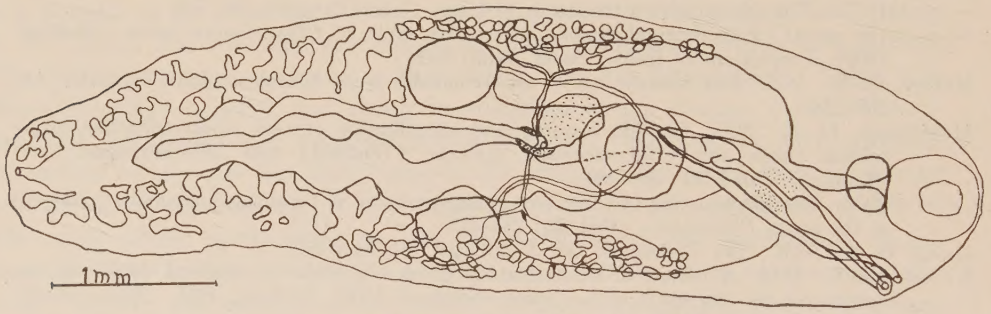


Fig. 1. Drawn from projection of the type specimen.

the specimens, five of which are to be deposited in the United States National Museum.

*Neorenifer crotali* sp. nov.

*Host: Crotalus atrox atrox* Baird and Girard.

*Location:* Lower lung.

*Description:* Cuticular spines absent. Tube-like integumentary glands numerous in anterior half of body. Body ovate, slightly more pointed at the posterior end and widest in the region of the testes. Average length 4.89 mm (3.50–6.22), average width 1.31 mm (0.91–1.70). Ratio of length to width 3.7 : 1. Diameter of acetabulum 0.47 mm and of oral sucker 0.42 mm with a ratio of 1.2 : 1. Length of esophagus 0.37 mm. Ratio of body length to length of esophagus 15.2 : 1. Length of cirrus pouch 1.37 mm. Ratio of body length to that of cirrus pouch 3.5 : 1. The cirrus pouch completely incloses the seminal vesicle and extends dorsal to the right anterior part of the acetabulum. The seminal vesicle is roughly S-shaped or slightly spiraled and is joined to the cirrus organ by a slender duct. The prostate is indistinct. The genital pore opens on the left margin and at the level of or slightly anterior to the level of the oral sucker-pharynx juncture.

The ovary is dextrosubmedian and partially overlaps the acetabulum. The diameter of the ovary is 0.24 mm. The shell gland is poorly defined and consists of a glandular tissue around the proximal end of the uterus. The eggs are oval in shape, averaging 0.04 mm in length and 0.02 mm in thickness. The testes are opposite, and slightly posterior to the midregion of the body.

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[1] Contribution from the Zoological Laboratory, University of Oklahoma.

[2] Now Acting Refuge Manager, Havasu Lake National Wildlife Refuge.

The diameters of the testes, 0.36 mm. The testes vary from a spherical to slightly ovate, but not lobed, condition. The vasa efferentia join just before entering the cirrus pouch. The *vitellaria* are marginal in the central third of the body and are not divided into anterior and posterior groups.

The excretory vesicle extends from the slightly subterminal excretory pore to the level of the testes where it connects with two large lateral tubules. It is profusely lobed, the lateral lobes being highly convoluted.

## DISCUSSION

The new species is more closely related to *N. septicus* (McCallum, 1921) and *N. serpentis* Schmidt and Hubbard, 1940, than to any others, accepting the opinion of Byrd and Denton (1938) that *R. ophiboli* McCallum, 1921, is a synonym of *N. septicus*. It differs from both *N. septicus* and *N. serpentis* in the host and the position which it occupies in the host. It differs from *N. septicus* in having a larger pharynx in relation to the size of the oral sucker (ratio of 1.7:1 as compared to 2.5: 1) ; in having a shorter esophagus, (ratio to body length of 1: 15.2 as compared to 1: 9.3) and in having the genital pore marginal and much more anteriorly located. It differs from *N. serpentis* in having a lobed excretory vesicle, in having a marginal genital pore, a longer esophagus, and a less distinct shell gland.

In addition to *N. septicus,* and *N. serpentis* fourteen other species belonging to this genus are known to the author. Information on *N. grandispinus* was taken from Manter (1943) and on the others which follow from Byrd and Denton (1938). The absence of cuticular spines distinguishes the new species from *N. wardi* (Byrd, 1936), *N. kansensis* (Crow, 1913), *N. formosum* (Nicoll, 1911), *N. elongatus* (Pratt, 1903), *N. zschokkei* (Volz, 1899), *N. validus* (Nicoll, 1911), *N. sauromates* (Poirier, 1885), *N. georgianus* Byrd and Denton, 1938, *N. heterodontis* Byrd and Denton, 1938, *N. glandularis* Byrd and Denton, 1938, and *N. grandispinus* (Caballero, 1938). It is distinguished from *N. acetabularis* (Crow, 1913) and *N. aniarum* (Leidy, 1890) by the continuous arrangement of the vitellaria and from *N. orula* (Talbot, 1934) by the sinistral position of the genital pore.

Certain striking similarities to both *N. glandularis* and *N. grandispinus* are apparent but it is necessary to keep it distinct from these species because of the absence of cuticular spines.

Schmidt and Hubbard (1940) have pointed out that *N. serpentis* may be a synonym of *N. septicus*. It seems definitely to be distinct, however, because it lacks the lobed excretory vesicle apparent in *N. septicus*. The species described here seems more likely to be a synonym of *N. septicus* although the description of the latter by McCallum makes comparison of other specimens with it difficult. The marked differences apparent in the length of the esophagus, level of the genital pore, size of the pharynx in relation to that of the oral sucker and in the position in the host as well as their being in widely separated host species seem to justify the establishment of a new species.

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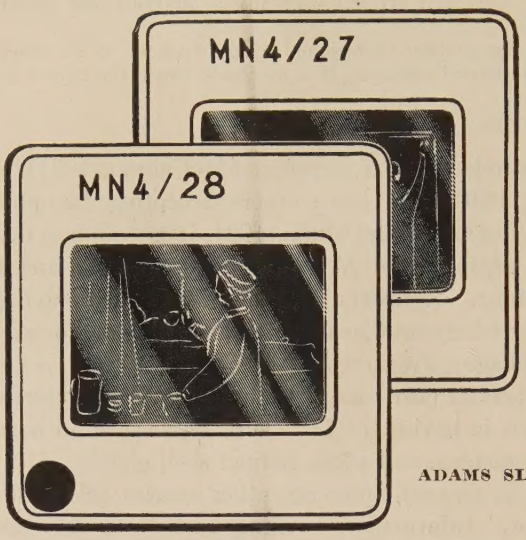


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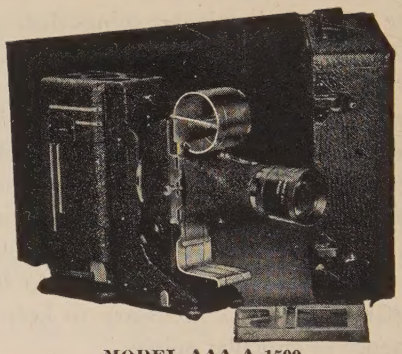

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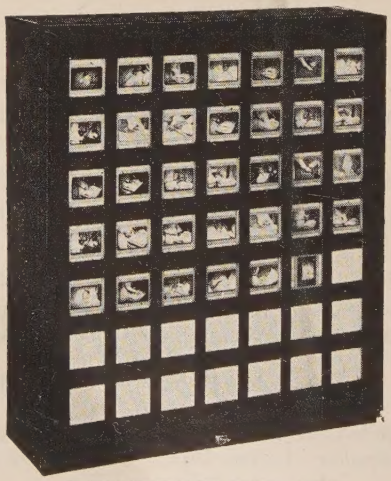

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